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EFFECT OF NARROW CARBON-NITROGEN RATIO AND OF NATURALLY OCCURRING TANNINS IN DECOMPOSING PLANT MATERIALS UPON THE PRODUCTION OF MUCUS

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Effective use of decomposed vegetable matter in soil management depends upon its fulfilling two primary objects; first, it must supply its nitrogen, most of which is present in the organic form, by a gradual process of mineralization; second, it should contribute to the good tilth of the soil through the help of the mucuslike bodies which develop during its decomposition. Nitrogen metabolism in composts and related materials has been studied thoroughly, and a number of papers are still devoted to this subject; but the other aspect of the problem relating to the production of mucus during the decomposition of plant materials is still young and needs close examination.

In previous communications (4, 5, 6) on this subject it has been remarked that the presence of mucus in manures derived from the decomposition of vegetable materials has a twofold significance. On the one hand, it has important applications in agricultural practice; and, on the other, the production of this colloidal material is an indication that decomposition has occurred under optimal conditions. These optimal conditions include aerobic fermentation, abundance of microbial tissue, growth of mucus-producing organisms, and an alkaline reaction. Microorganic bodies are rich in protein, the carbon-nitrogen ratio varying between 5.5 and 15.8 (2). Moreover, proteins are colloidal in nature, and some, like caseinogen and peptone, form a sticky mass when wetted with water. These observations led the author to study the problem of stickiness with plant materials naturally rich in protein.

In former publications (4, 5, 6), the plant materials used for decomposition were of a low protein content with a wide carbon-nitrogen ratio such as oat and ragi (*Eleusine coracana*) straws. In the present investigation, however, a number of plant materials of relatively high protein content covering a wide range of carbon-nitrogen ratios were subjected to fermentation to determine whether the property of mucus production is in any way dependent upon the nature of the plant tissue. The amount of mucus produced was measured by the apparatus as described in the following section.

¹ The author's thanks are due to R. V. Norris, director of the institute, for permission to publish this paper, and to T. Eden, agricultural chemist, for the facilities provided for the conduct of this investigation and for his valuable criticism and advice.

APPARATUS FOR MEASURING STICKINESS

The apparatus consists of a pillar and fixed beam, in appearance T-shaped after the fashion of a chemical balance on a baseboard. Each of the extremities of the beam carries a pulley. Below one pulley are two square plates, between which is placed the material whose adhesiveness is to be measured. The lower of these, which is the larger, slides into permanent guard rails which keep it firmly in position. The upper plate rests on the lower one and is small enough to clear the guard rails by about a quarter inch all round. To its center is welded a small ring which, by means of hook and cord passing over the pulleys, connects to a metal container capable of holding about 2 kgm. of lead shot. When all parts are in position the container hangs free above the base board. In addition, there is provided a slotted and molded lead block weighing 1,600 gm. which fits over the top plate. The purpose of this is to exert a standard and uniform pressure on the material that is sandwiched between the two plates during the preparatory stages of the test. The plates should be sufficiently thick not to bend under the forces applied, and the lower plate must be firmly fixed.

Any material to be tested for stickiness is first freed from gritty material and then cut as finely as possible with a pair of scissors, after which it is well mixed with a spatula to give a representative sample. One-gram lots are then uniformly sandwiched between the two plates, by pressing with the weight for 15 seconds, and are dried in an oven at 100°C. Uniform and complete drying was found to occur in 2 to 3 hours. Since the area of the manure and its thickness depend upon the time and force with which the two plates are pressed, and the breaking pull to separate the two plates is dependent upon the distance between them, it is essential that the weight and time of pressing be kept constant so as to obtain comparable results.

When the plates are ready for measuring the pull, the lower plate is gently slid in, and the cord over the pulley is connected at its respective ends to the hook in the plate and to the container. Lead shots are gradually poured in until the top plate parts instantaneously from the rest of the system. The pull is then obtained by subtracting the weight of the top plate from that of shot weight and container. A mean diameter of the block of sandwiching material is obtained by measuring 5 or 10 diameters in different positions. This is necessary because the block is never completely regular in form. Having measured the pull in grams and the area, the force per unit area can be calculated on the basis of dry weight. The value thus obtained is a measure of the stickiness in the material.

On statistical examination of the data it was found that the method gave significant differences between highly and moderately sticky samples and that the magnitude of the error varied inversely as the stickiness.

The foregoing test should always be conducted on fresh material. It was observed that the pull on a sample which was dried and then wetted was considerably lower than that on the fresh one because of the irreversible nature of

the colloids. It is also very necessary that the plates should not be left untested for any length of time, as the dried material begins to absorb moisture and the result thus obtained is lower than the true value. For a similar reason it is advisable to continue the test until the plates separate instantaneously.

EXPERIMENTAL

The physical test as described was conducted on the various fermented materials as given below.

The dozen plant tissues were allowed to undergo aerobic fermentation in the presence of a mixed natural flora at 30° for 35 days with the following changes in the source of available nitrogen and adjustment of the reaction:

Without any external source of nitrogen.

In the presence of ammonium sulfate, which may be expected to develop an acid reaction during fermentation.

In the presence of sodium nitrate, which develops an alkaline reaction during fermentation.

Adjustment of the final reaction with sodium hydroxide, sulfuric acid, and tannic acid.

Technic and methods

Twenty grams of oven-dry material of known nitrogen content were fermented aerobically in bottles with a fixed amount of inoculum from a compost. Nitrogen was supplied in the two forms to the extent of 1 gm. per 100 gm. of material, and the moisture was adjusted to about 70 per cent.

Organic carbon in the original material was determined by the method of Robinson, McLean, and Williams (3); total nitrogen was determined by the usual Kjeldahl method; and pH measurements were made on the compost water extracts by the electrometric method.

Results

Composition of plant materials. Table 1 contains the analytical data for the carbon-nitrogen ratios of the different plant materials examined in this investigation. All the materials except maana grass, grevillea, cane reed, and paddy straw have a carbon-nitrogen ratio which is very near to the one obtained by composting a vegetable material. In fact, most of the materials are used as green manures on tea estates in Ceylon and on account of their high nitrogen content are incorporated with the soil without any pretreatment.

Stickiness with respect to loss of dry matter and final reaction. Table 2 shows the losses of dry matter during fermentation of the various materials, their respective reactions, and the results of the physical test. The losses of dry matter with their effect on the nitrogen transformation will form a subject of another communication. It is intended here to discuss only the problem of mucus production with respect to the loss suffered by any plant material and the reaction it developed subsequent to its fermentation under the three different treatments. The general trend of results, with a few exceptions, follows the chief factors established in an earlier paper (4). The degree of stickiness,

as shown by the figures, varied with the final reaction and the degree of decomposition for a particular material. Ammonium sulfate rots are invariably less sticky than rots without any treatments, which in their turn are generally

TABLE 1
Carbon-nitrogen ratios of unfermented materials

MATERIAL	CARBON	NITROGEN	C/N RATIO
	<i>per cent</i>	<i>per cent</i>	
(<i>Tithonia diversifolia</i>) Sunflower.....	36.91	3.37	10.96
<i>Tephrosia vogelii</i>	43.40	3.46	12.56
Refuse tea.....	42.73	3.97	10.75
(<i>Erythrina lithosperma</i>) Dadap.....	40.62	2.54	15.97
<i>Gliricidia sepium</i>	40.48	2.74	14.77
(<i>Andropogon nardus</i>) Maana grass.....	39.92	1.37	29.07
(<i>Camellia sinensis</i>) Tea leaf.....	42.63	3.19	13.38
<i>Grevillea robusta</i>	48.78	1.04	47.10
Weeds.....	34.99	2.01	17.43
Fern.....	41.01	1.79	22.88
(<i>Oriza sativa</i>) Paddy straw.....	34.59	0.78	44.12
(<i>Pennisetum</i> sp.) Cane reed.....	36.92	0.96	38.50

TABLE 2
Loss of dry matter during fermentation of original material, pH, and results of the physical test

MATERIAL	AMMONIUM SULFATE TREATMENT			NO TREATMENT			SODIUM NITRATE TREATMENT		
	Loss of dry matter	pH	Physical test	Loss of dry matter	pH	Physical test	Loss of dry matter	pH	Physical test
	<i>per cent</i>		<i>gm.</i>	<i>per cent</i>		<i>gm.</i>	<i>per cent</i>		<i>gm.</i>
Sunflower.....	20.9	7.89	577	41.6	8.15	1,116	36.9	9.03	2,742
Tephrosia.....	31.0	7.76	715	38.5	8.27	846	28.4	8.68	1,325
Refuse tea.....	21.1	7.49	33.4	7.66	166	25.4	8.09	329
Dadap.....	20.0	7.30	416	51.3	8.39	480	24.3	9.10	730
Gliricidia.....	40.6	8.27	1,150	48.4	8.58	1,720	38.7	9.12	1,212
Maana grass.....	33.7	5.79	228	7.23	40.3	8.87	313
Tea leaf.....	31.5	7.71	329	35.6	8.16	252	30.0	8.72	287
Grevillea.....	7.1	5.53	210	6.92	5.3	7.26	281
Weeds.....	54.1	7.59	275	51.0	8.01	846	43.1	9.83	>1,585
Fern.....	24.4	4.52	21.9	5.51	32.5	7.73
Paddy straw.....	24.9	6.73	594	15.4	8.73	2,021
Cane reed.....	34.7	4.44	37.4	8.58	3,306

less sticky than sodium nitrate rots. This is ascribed to the changes in reaction. For instance, sunflower and tephrosia, although they suffer a greater loss under no treatment, are less sticky than when treated with sodium nitrate. The richness in protein of a plant material, therefore, seems to have an effect

on the degree of rotting but does not seem to have any direct influence on the quantity of mucus. Additional doses of nitrogen in the form of ammonium sulfate and sodium nitrate have actually lowered the losses of dry matter and have affected the stickiness by disturbing the reaction, as indicated in the table. It is noteworthy that the pH of ammonium sulfate rots of plant materials rich in protein has not fallen to the same extent as has that of materials like maana grass, grevillea, and fern, which are naturally poor in protein. The pH of the former is on the alkaline side rather than on the acid side, which is the case with the latter.

There are two exceptions to the general trend of results: first, materials such as fern and dadap which have resistant midribs that decompose less rapidly than the leafy materials and that interfere with the physical test when the more succulent parts are fully rotted; second, tea leaf or refuse tea which behaves in a quite anomalous manner, although the physical consistency of the rot in no way interferes with the stickiness test. These will receive more detailed consideration later in this paper.

Effect of modification of reaction on the stickiness. Two types of compost were selected—gliricidia with a high degree of stickiness, and tephrosia fermented with ammonium sulfate with moderate stickiness—to demonstrate the effect of reaction and to obtain a correlation between stickiness and reaction. The pH of 10 was adjusted with sodium hydroxide. This particular reaction was chosen because the highest figure hitherto obtained for stickiness was found in a rot supplied with sodium nitrate which had attained finally that high degree of alkalinity. Reaction on the acid side was adjusted with sulfuric acid and tannic acid. Tannic acid, as the most easily available tannic substance, was used particularly because refuse tea and tea leaf, which are rich in tannins, behaved in an exceptional way, as has been pointed out. Table 3 records results with different pH adjustments. The value for stickiness increased with alkalinity and fell with acidity. The effect of tannic acid was contrary to expectation, inasmuch as the resultant fall in pH had no appreciable effect on stickiness.

The queer behavior of tannic acid may be ascribed to two factors: first, increasing amounts of tannic acid have to be mixed with the compost to lower the pH, and second, tannic acid itself produces a sticky paste when mixed with water, with the result that the drop in stickiness due to acidity is counter-balanced by the stickiness introduced by tannic acid. This suggests that the tannins present in complex forms in the tea leaf have a fundamentally different action during the process of decomposition from that of a mere addition of commercial tannic acid, which when mixed with a compost forms only a mechanical mixture.

Effect of tea leaf and Gordonia leaf and their water extracts on the production of stickiness. Despite the fact that commercial tannic acid did not produce an effect similar to that recorded for tea, it was regarded as probable that the tannic complex in the tea, closely associated as it is with the fermentable

carbohydrate and protein bodies, might be responsible for the exceptional behavior of tea leaf decomposition. That the presence of tannin does not impede the decomposition is shown by the figures for dry matter loss and reaction values. But it does appear to change the decomposition qualitatively. The two most obvious ways in which this might occur are, first, by a selective control of microflora, whereby the organisms responsible for the development of mucus were inhibited, and second, by a precipitation or similar alteration in the condition of the mucus when formed.

In order to test these suggestions, gliricidia, which, when decomposed alone, produces the stickiest compost, was fermented along with tea leaf containing 7.93 per cent tannin in one case, and with the water extract of tea leaf in an-

TABLE 3
Effect of modification of the pH on the production of stickiness

MATERIAL	LOSS OF DRY MATTER	ON FERMENTATION		ON ADJUSTMENT	
		pH	Physical test	pH	Physical test
	<i>per cent</i>		<i>gm.</i>		<i>gm.</i>
Gliricidia	48.4	8.58	1,720	10.0—NaOH	1,786
				7.5 } H ₂ SO ₄	1,288
				5.7 }	942
				4.8 }	625
				3.5 }	520
Tephrosia + (NH ₄) ₂ SO ₄	31.0	7.76	715	10.0 } NaOH	1,239
				9.2 }	1,170
				8.3 }	766
				6.25 } H ₂ SO ₄	700
				4.00 }	606
Gliricidia	48.4	8.58	1,720	7.00 } Tannic acid	1,544
				5.85 }	1,413
				4.50 }	1,507

other. Further, in order to determine whether tannin could alter mucus already formed, a series was set up in which the addition of extract was delayed for 3 weeks, at which time mucus formation would be well advanced. A similar series was carried out using a *Gordonia* (probably *imbricata*), another plant having a marked tannin content (15.31 per cent). The schedule of the fermentations was accordingly as follows: (a) Gliricidia alone, (b) Gliricidia and tea leaf, (c) Gliricidia and tea leaf extract, (d) Gliricidia (3 weeks' compost) and tea leaf extract, (e) Gliricidia and *Gordonia imbricata*, (f) Gliricidia and *Gordonia* extract, (g) Gliricidia (3 weeks' compost) and *Gordonia* extract, and (h) *Gordonia*. Fermentation in these bottles went on for a total of 45 days instead of 35 as in the previous experiments. Peptone was added as a

source of available nitrogen to the last bottle, as *Gordonia* has a positive nitrogen factor. *Gordonia*, like the tea leaf, produced no stickiness, as will be seen from table 4.

Introduction of tea leaf and *Gordonia* at the very start of the experiment would show the effect of these materials on the stickiness normally produced by *gliricidia* while fermenting independently. The use of water extracts of equal amounts of the two materials instead of their direct incorporation would suggest the action of their active constituents, which are tannin in both cases. It has been observed that the major part of the water extract of tea leaf prunings consists of tannin with negligible amounts of protein. Water will no doubt extract some hemicelluloses, starch, and other soluble carbohydrates, but these constituents will help the production of mucus rather than inhibit it. Introduction of such an extract after *gliricidia* compost had advanced in age and thus attained a major part of its normal stickiness would

TABLE 4
Effect of tea leaf and Gordonia imbricata and their respective water extracts on the production of stickiness

MATERIAL	LOSS OF DRY MATTER	pH	PHYSICAL TEST
	<i>per cent</i>		<i>gm.</i>
<i>Gliricidia</i>	52.6	7.85	1,507
<i>Gliricidia</i> + tea leaf	43.9	8.09	362
<i>Gliricidia</i> + tea leaf extract	51.3	8.62	365
<i>Gliricidia</i> (3 weeks) + tea leaf extract	53.1	8.03	1,186
<i>Gliricidia</i> + <i>Gordonia</i>	41.9	7.58	357
<i>Gliricidia</i> + <i>Gordonia</i> extract	51.8	7.75	324
<i>Gliricidia</i> (3 weeks) + <i>Gordonia</i> extract	58.6	8.70	1,910
<i>Gordonia</i>	16.8	8.70

at once indicate the modifying effect of tannin on mucus stability. In other words, if no stickiness is recorded in bottles (b), (c), (e), and (f), it means, in effect, that tannins modify the flora and have a toxic action on the organisms responsible for the production of mucus. If the stickiness obtained with *gliricidia* alone in bottle (a) is reproduced with the treatments in (d) and (g), it means that once the mucus has been synthesized by the microorganisms, tannins have practically no effect on the production of stickiness. That such is the case is demonstrated by data presented in table 4.

The adverse effect of tea leaf and its active constituent is thus due to the selective toxicity of tannin on the microflora. The parallel case of *Gordonia*, which, too, is a tannin-containing material, confirms the fact that tannin is the essential disturbing factor in the production of stickiness. At one time it was thought that this inhibition process might be a chemical one, as mucus which contains protein bodies might be precipitated in the presence of tannins of the plant materials. This does not appear to be so for two reasons: first,

the presence of tannin-rich materials from the earliest stages of decomposition does not allow the normal growth of mucus in gliricidia; second, mucus once synthesized by gliricidia was not affected by the addition of water extract after 3 weeks. Had it been a mere case of precipitation, the tannins in this extract ought to have modified the stickiness by combining with the mucus protein. It is, therefore, reasonable to suppose from the results in table 4 that the nonproduction of stickiness in tea leaf and *Gordonia* composts is essentially a microbial process rather than a chemical one. Such toxicity and specificity of tannin to the course of microorganic development during fermentation of plant materials find support from the work of several investigators, as summarized by Nierenstein (1). Tea leaf fermented for different periods up to 365 days also failed to indicate any stickiness.

SUMMARY

Decomposition of a number of plant materials in the presence of sulfate of ammonia and sodium nitrate and without any treatment has been studied with regard to the question of stickiness.

Initial high protein content of a plant material has no direct bearing on the amount of stickiness but indirectly affects it through the degree of decomposition. A difference thus seems to exist between the synthesized microbial protein and the original plant protein with respect to the production of stickiness.

Provided the reaction developed is suitable, production of stickiness during fermentation appears to be independent of the nature of the plant material, with the exception of plant materials containing tannin.

The presence of tannins in refuse tea, tea leaf prunings, and *Gordonia* has been shown to exert a toxic effect on the microflora concerned in the decomposition, and the inhibition of mucus production seems to be a microbial process rather than a chemical one.

Production of stickiness will indicate the reaction and, in the light of previous work, the nature of fermentation (whether aerobic or anaerobic) of a fermenting vegetable heap.

REFERENCES

- (1) NIERENSTEIN, M. 1934 *The Natural Organic Tannins*. J. & A. Churchill, Ltd., London.
- (2) NORMAN, A. G. 1933 The biological decomposition of plant materials: VIII. The availability of the nitrogen of fungal tissues. *Ann. Appl. Biol.* 20: 146-164.
- (3) ROBINSON, G. W., McLEAN, W., AND WILLIAMS, R. 1929 The determination of organic carbon in soils. *Jour. Agr. Sci.* 19: 315-324.
- (4) SHRIKHANDE, J. G. 1933 Production of mucus during the decomposition of plant materials: I. The effect of environmental conditions. *Biochem. Jour.* 27: 1551-1562.
- (5) SHRIKHANDE, J. G. 1933 Production of mucus during the decomposition of plant materials: II. Effect of changes in the flora. *Biochem. Jour.* 27: 1563-1574.
- (6) SHRIKHANDE, J. G. 1936 The production of mucus during the decomposition of plant materials: III. The effect of partially aerobic and anaerobic conditions. *Biochem. Jour.* 30: 1789-1794.

THE INFLUENCE OF AMINO ACIDS AND PROTEINS ON NITROGEN FIXATION BY AZOTOBACTER CHROOCOCCUM¹

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Azotobacter species differ from most other microorganisms in that they are capable of obtaining nitrogen from the air. This ability to gather nitrogen is dependent upon appropriate conditions. According to some writers *Azotobacter* obtains little nitrogen from the air in the presence of combined nitrogen. Mockridge (28) has even gone so far as to say: "It is a well known fact that when supplied with soluble nitrogen, *Azotobacter* does not fix atmospheric nitrogen until the available nitrogen has been consumed." Lipman (24), Stranak (34), Hills (17), Heinze (16), Stoklasa (33), and Greaves and Nelson (13) found, however, that small quantities of nitrates stimulated *Azotobacter*, whereas larger quantities discouraged nitrogen fixation, since the organisms live on the nitrates (36). This is the case whether the nitrates are added to the soil or to the solution in which nitrogen is being fixed.

Decreases in the activities of both aerobic and anaerobic nitrogen fixers have been reported where nitrates have been applied to the soil (6). Coleman (5) considers this action to be the result of two factors: (a) direct toxic action of the salt and (b) antagonism of other organisms favored by the salt. This theory appears to be borne out by the fact (13) that the addition of mannite alone to some soils decreases their nitrogen-fixing powers as determined by the tumbler method, but its application in connection with 0.084 per cent of sodium nitrate, calcium nitrate, magnesium nitrate, or manganese nitrate increases the nitrogen-fixing powers. When 2 per cent of dried blood was applied to a soil, it increased the nitrogen-fixing power, but when applied in conjunction with 0.084 per cent of the various nitrates there was a loss of nitrogen.

Zoond (37) found that increasing concentrations, over 0.003 per cent of nitrate, amino acid, or peptone, decreased nitrogen fixation by *Azotobacter*. Sterile, unheated plant extracts when applied in moderate amounts increased fixation.

Nitrates and ammonium sulfate are rather effective in stimulating nitrogen

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fixation when *Azotobacter* is grown in connection with the cellulose ferments (27). Even here, however, large quantities have been found to reduce this power (22, 25). In pure cultures ammonium sulfate (23, 24) seriously retards nitrogen fixation, whereas the nitrogen of humus, even in large quantities, appears to have no serious retarding influence.

Hanzawa (14), Löhnis and Green (26), Murray (29), Brown and Allison (3), Fulmer and Fred (9), Greaves and Nelson (13), and Richards (31) all found that organic manures increased the activities of nitrogen fixers, the carbohydrate content often acting as a source of energy (24). Corn roots and stalks (7), oak leaves, lupine, alfalfa, maple leaves, and pine needles—in short, the tissues of most plants—stimulate nitrogen fixers (19, 29). Apparently the tissues from the nonlegumes are more efficient than those from the legumes (3). When the nitrogen content of the soil, even that due to organic matter, exceeds a certain value, nitrogen fixation is decreased (2, 18). Legumes growing (12) in a soil may so reduce its nitrate content that *Azotobacter* organisms are stimulated.

Reed and Williams (30) studied the effect of various organic compounds, including many which are toxic to higher plants, on the growth of *Azotobacter* and found that nitrogen fixation was only slightly influenced by most of the compounds used. Even in concentrations fatal to certain higher plants, many of the compounds depressed fixation only slightly. Certain of the compounds, especially urea, glycine, formamide, and allantoin, at concentrations of 500 p.p.m. were particularly active in depressing nitrogen fixation. It was suggested that this was due not to toxic effects but rather to the utilization of the compounds by *Azotobacter*.

Burk and Lineweaver (4) found that the quantity of readily available combined nitrogen required to inhibit nitrogen fixation by *Azotobacter* was 0.5 mgm. per 100 cc. of solution.

Fuller and Rettger (8) determined the influences of a large number of nitrogen compounds on nitrogen fixation by *Azotobacter* and noted that most of the nontoxic compounds did not influence fixation to any great extent. Certain of the compounds such as aspartic acid, cysteine hydrochloride, glyocoll, creatine, creatinine, and urea particularly inhibit nitrogen fixation.

Proteins are decomposed in the soil by microorganisms with the production of various amino acids. Jodidi (20) summarized the early work on the occurrence and kind of amino acids in soil, and in a later paper he (21) reported the quantity and quality of the various nitrogen-carrying constituents occurring in variously treated soils. Headden (15) found an average of 0.04 per cent of amino nitrogen in some Colorado soils. Many of the amino acids have been obtained from soil, but the quantity and the kind vary with different soils (32). Thompson (35) found that *Azotobacter vinelandii* utilized small quantities of some amino acids, but these were either not used or used only in small quantities by *Azotobacter chroococcum*. In those cases where amino acids were utilized, nitrogen fixation was depressed, whereas in other cases where they were not utilized, the amino acids acted as a stimulant to nitrogen fixation.

STATEMENT OF PROBLEM

It is evident from the literature that *Azotobacter* does fix atmospheric nitrogen in the presence of combined nitrogen. The specific influence of this combined nitrogen varies with the kind and the quantity present. It is also evident that amino acids occur in soil and that little work has been done to learn their influence on *Azotobacter chroococcum*. This paper reports the results of a study of the influence of varying quantities of the following amino acids and proteins on nitrogen fixation by *Azotobacter chroococcum*: glycine, leucine, dl-isoleucine, dl-valine, isovaline, l-aspartic acid, glutamic acid, dl-lysine, d-arginine, dl-methionine, cystine, tyrosine, phenylalanine, l-tryptophane, hydroxyproline, l-histidine, l-proline, casein, albumin, and gelatin.

METHODS

A strain of *Azotobacter chroococcum* isolated from the college farm soil was used in this work. The organism was cultured in a synthetic medium of the following composition:

KH ₂ PO ₄	0.02 per cent	NaI.....	40 p.p.m. iodine
MgSO ₄	0.02 per cent	MnCO ₃	40 p.p.m. manganese
NaCl.....	0.02 per cent	CaCO ₃	1.0 per cent
CaSO ₄	0.01 per cent	Mannitol.....	1.5 per cent
FeSO ₄	50 p.p.m. iron	Distilled H ₂ O....	1,000 cc.

All chemicals used were of the highest purity. To this basic medium were added the amino acids in quantities ranging from 5 to 150 p.p.m. The quantity of proteins added carried from 5 to 150 p.p.m. of nitrogen.

The medium, in 100-cc. portions, together with the varying quantities of the amino acids and proteins, was distributed in 500-cc. Erlenmeyer flasks. The flasks were autoclaved for 15 minutes at a pressure of 15 pounds and then inoculated with *A. chroococcum*. The inoculated flasks together with sterile blanks were incubated at 28 to 30°C. for 3 weeks. At the end of the incubation period the nitrogen was determined by the Kjeldahl method (1, p. 8). The quantity of nitrogen found in the blanks together with the amount added in the amino acid was subtracted from the total and the balance reported as nitrogen fixed. The quantity of nitrogen fixed by *A. chroococcum* in the flasks containing only the basic medium was from 9 to 12 mgm. and in the results is given as 100. This is taken as a basis for reporting the results obtained in the presence of the various amino acids. Each reported result is the average of five or more closely agreeing determinations.

EXPERIMENTAL

The results obtained when *A. chroococcum* was grown in the presence of the aliphatic mono-amino-monocarboxylic acids are given in figure 1. Dl-isoleucine is an active stimulant for *A. chroococcum*. In the presence of 100 p.p.m. of dl-isoleucine *A. chroococcum* fixes 75 per cent more nitrogen than it does in its absence. Even at a concentration of 150 p.p.m. isoleucine, nitrogen

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fixation is materially increased. Leucine, glycine, and dl-valine all slightly increase nitrogen fixation, but the concentration at which stimulation occurs varies with the different amino acid. Glycine stimulates most markedly when present in a concentration of 20 p.p.m., whereas dl-valine is most active at 50 p.p.m. Isovaline is apparently without effect. From these results it is highly improbable that any of these amino acids would occur in soil in sufficient quantities to retard nitrogen fixation by *Azotobacter*, and they may at times increase fixation. Furthermore the presence of amino acids in soil may be one of the reasons why nitrogen fixation is greater in soil than in laboratory media not containing the amino acids. There is nothing in these results to bear out the contentions of Reed and Williams (30) or Thompson (35) that glycine, when present in small quantities, depresses nitrogen fixation by

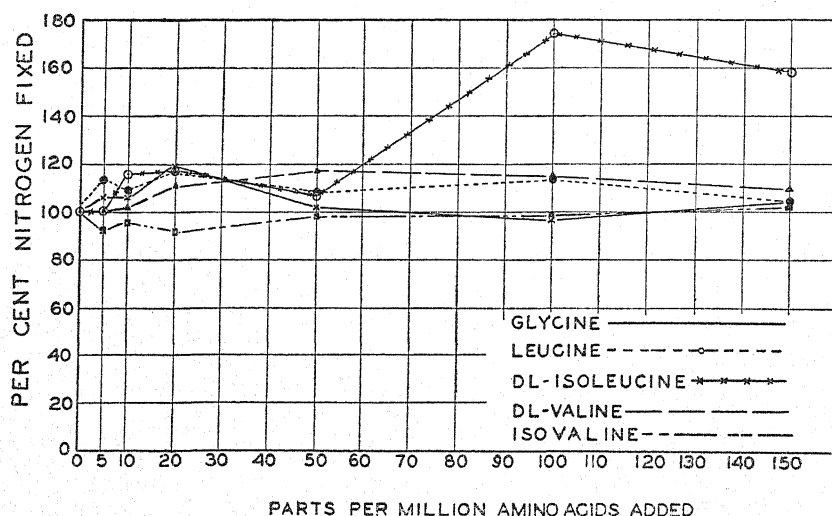


FIG. 1. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF GLYCINE, LEUCINE, dl-ISOLEUCINE, dl-VALINE, AND ISOVALINE

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

furnishing a ready supply of combined nitrogen. The results, however, would be different where comparatively large quantities of the amino acids are present. Hence, it may be concluded that leucine, glycine, dl-valine and dl-isoleucine stimulate nitrogen fixation by *A. chroococcum*, whereas isovaline is without effect.

The results obtained with the two aliphatic mono-amino-dicarboxylic acids are given in figure 2. Both l-aspartic acid and glutamic acid increase nitrogen fixation by *A. chroococcum*. The two are very similar in their effects; neither becomes toxic even at concentrations of 150 p.p.m. It is probable that these amino acids in the concentrations in which they occur in the soil would increase nitrogen fixation and never would accumulate in concentrations sufficient to retard fixation by *Azotobacter*. Although small quantities of glutamic acid

are utilized by *A. chroococcum* (31), they do not retard the assimilation of atmospheric nitrogen.

The results obtained with the aliphatic diamino-monocarboxylic acids are given in figure 3. D-arginine is without effect on *A. chroococcum* until the

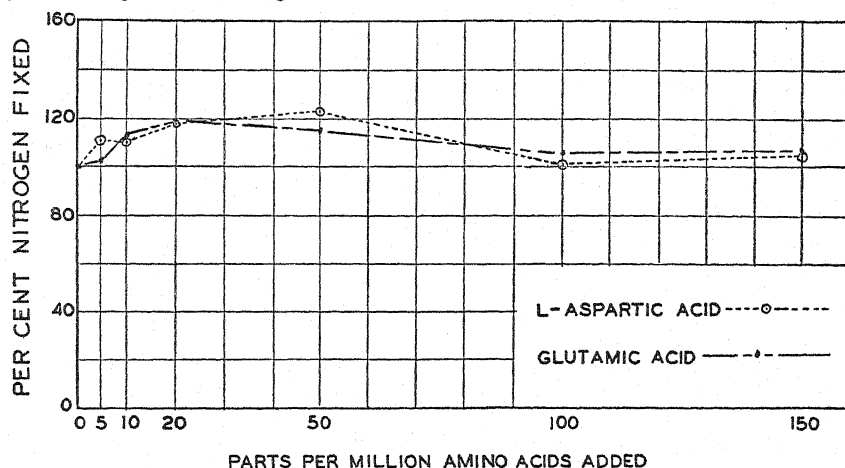


FIG. 2. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF L-ASPARTIC ACID AND GLUTAMIC ACID

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

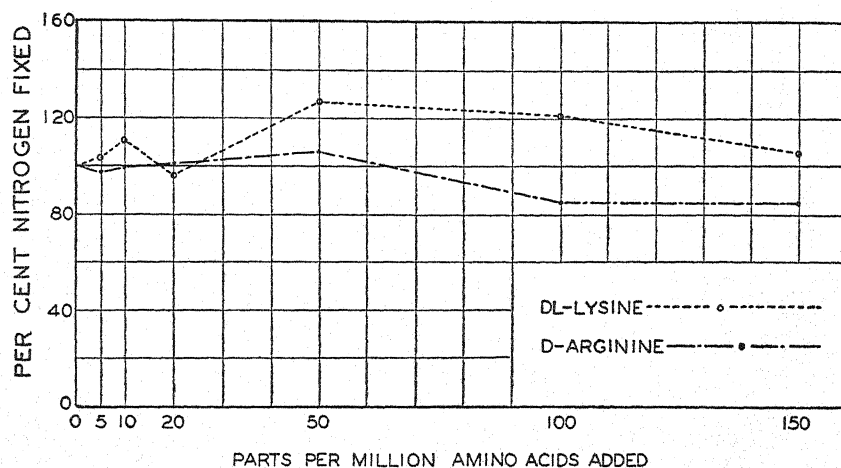


FIG. 3. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF dl-LYSINE AND d-ARGININE

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

concentration reaches 70 p.p.m.; above this concentration it becomes slightly toxic. Lysine, on the other hand, stimulates throughout the range of concentrations, the greatest stimulation occurring between the concentrations of 50 and 100 p.p.m.

The results obtained for the sulfur-containing amino acids are given in figure 4. Cystine carries a very acceptable form of sulfur for *A. chroococcum* (11), and the organism synthesizes appreciable quantities of glutathione which is used in its metabolic activities; hence it is not surprising to find that both cystine and dl-methionine stimulate nitrogen fixation by *Azotobacter*. In this respect they are of nearly equal value; both stimulate at the lowest concentration tested, and their actions nearly parallel each other throughout.

The results obtained for the two aromatic amino acids are given in figure 5. Tyrosine is a very active stimulant at a concentration of 5 p.p.m. In concentrations from 10 to 130 p.p.m. there is a slight stimulation and at a concentration of 150 p.p.m. it retards nitrogen fixation. Phenylalanine, on the other hand, is toxic at 5 p.p.m. and increases in toxicity as the quantity

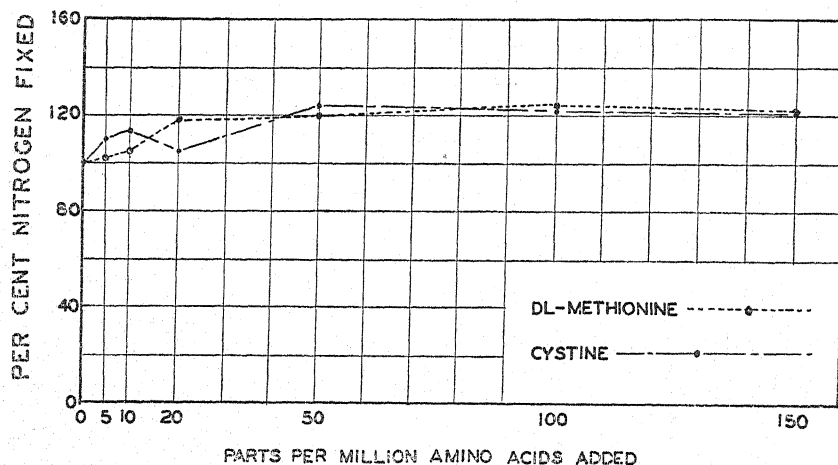


FIG. 4. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF dl-METHIONINE AND CYSTINE

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

increases. According to Thompson (35) tyrosine is not utilized by *A. chroococcum*. We have found it and phenylalanine to disappear from the medium, however, and when tested by the Hanke and Koerster's p-diazobenzenesulfonic acid reaction, phenol or cresol appears. It is probable that phenylalanine, to a limited extent, is transformed in the medium to tyrosine and then to phenol or cresol. Some of the phenylalanine is utilized by *Azotobacter* (35). These two factors account for its depressing effect. If phenylalanine is as toxic in soil as it is in solution, which is not probable, it may at times accumulate in the soil in concentration sufficient to retard nitrogen fixation.

The results obtained with l-tryptophane are given in figure 6. This amino acid stimulates throughout all the concentrations used. Hence, it is probable that *Azotobacter* neither uses it nor transforms it into the toxic indole.

The results obtained with the other three heterocyclic amino acids, hydroxy-

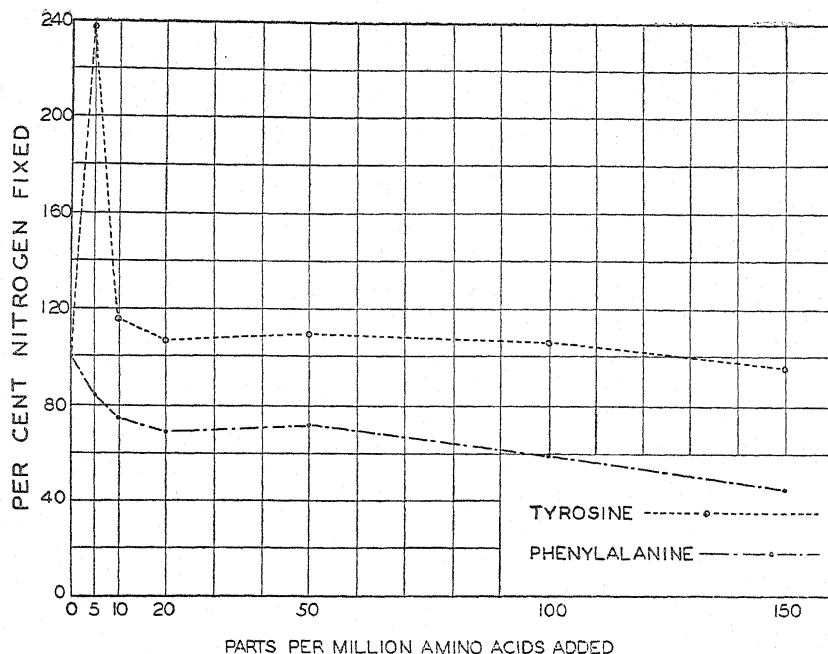


FIG. 5. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF TYROSINE AND PHENYLALANINE

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

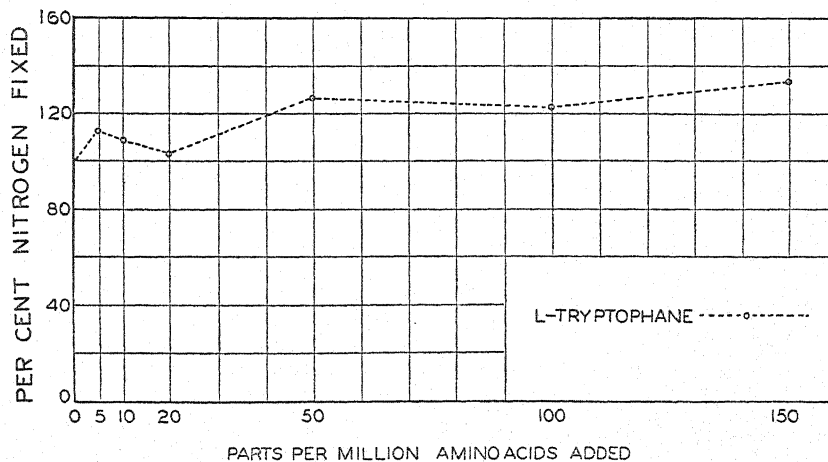


FIG. 6. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF L-TRYPTOPHANE

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

proline, l-proline, and l-histidine, are shown in figure 7. Hydroxyproline and l-histidine increase nitrogen fixation throughout the entire range of concentrations used, whereas l-proline, which lacks the hydroxyl group, is without

effect. It is safe to conclude that none of these compounds would accumulate in quantities sufficient to retard nitrogen fixation in normal productive soils, and it is probable that hydroxyproline and l-histidine may occur in the soil solution in quantities sufficiently great to increase nitrogen fixation by *Azotobacter*.

The results obtained where the three proteins, casein, albumin, and gelatin were added to the synthetic medium are given in figure 8.

Casein greatly stimulates nitrogen fixation by *A. chroococcum* when in solution. Even 5 p.p.m. is very effective, and it reaches its maximum effect at 20 p.p.m. In concentrations above this, it decreases the activity, but it is still a stimulant in concentrations of 150 p.p.m. In concentrations of 10

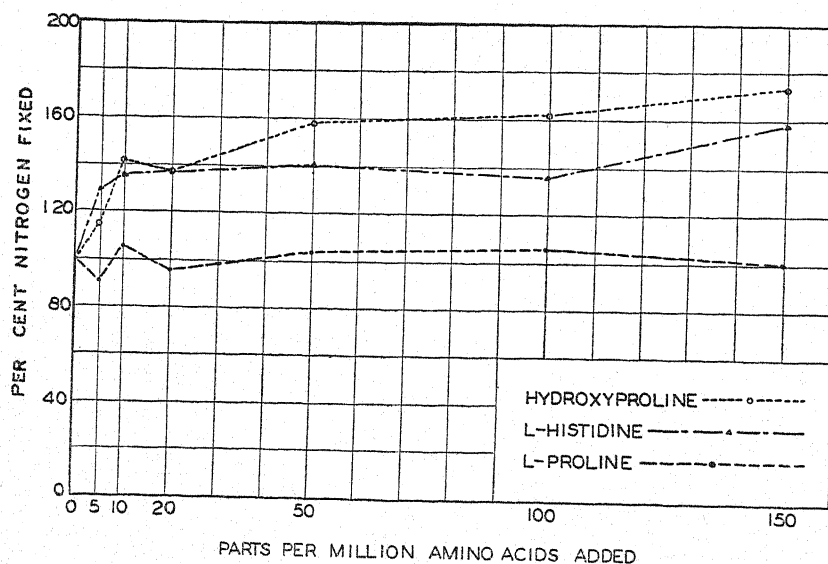


FIG. 7. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF HYDROXYPROLINE, L-HISTIDINE, AND L-PROLINE

The nitrogen fixed in the absence of amino acid is taken as 100 per cent

p.p.m. albumin increases nitrogen fixation 22 per cent; and in concentrations of 150 p.p.m., about 10 per cent. Gelatin, on the other hand, has no significant effect on nitrogen fixation in concentrations below 50 p.p.m., but in concentrations above 50 p.p.m. it rapidly increases in toxicity. In concentrations of 150 p.p.m. of gelatin, all fixation of nitrogen by *A. chroococcum* ceases.

This variation in action of the different proteins upon *Azotobacter* cannot be attributed to their difference in solubility, because all were soluble in the medium, nor can the retarding action of gelatin be attributed to the utilization by the *Azotobacter* of the nitrogen of the gelatin, for this organism cannot hydrolyze gelatin (8) and uses it to a very limited extent (35). Hence, gelatin must exert a direct toxic action on *A. chroococcum*. On the other hand, casein, albumin, and dried blood all increase nitrogen fixation both in soil (13) and in

solution. It is possible that the action is similar to that of other colloids (10), for nitrogen fixation is decidedly increased by the addition of either soil humus or humic acid. Soil extract and also extracts of plant tissues when added to Ashby's nutritive medium (10) increase nitrogen fixation by *Azotobacter*. When these extracts are ashed and the ash is added, part of the beneficial effect is lost; hence the action cannot be attributed wholly to the mineral concentration, but may be due to (a) the colloidal constituents, albumins, globulins, and casein, which increase the catalytic action of the iron (it has been demonstrated that colloidal iron is better for this purpose than iron in solution) or (b) other constituents, possibly vitamins, which may be carried by albumins and casein and which accelerated nitrogen fixation by *A. chroococcum*.

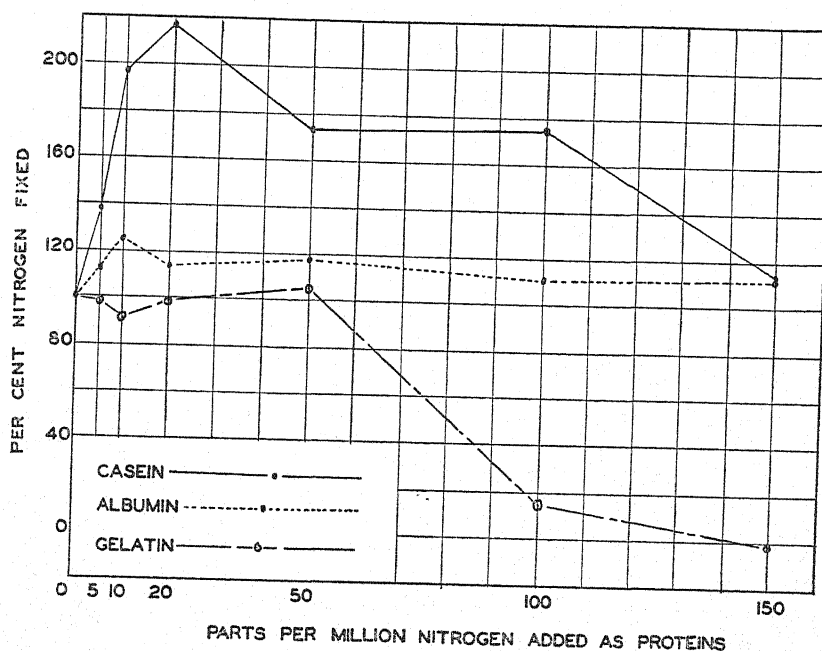


FIG. 8. PERCENTAGES OF NITROGEN FIXED IN THE PRESENCE OF VARYING QUANTITIES OF CASEIN, ALBUMIN, AND GELATIN

The nitrogen fixed in the absence of protein is taken as 100 per cent

In order to determine whether any accessory substances soluble in water were responsible for the stimulation, casein was dialyzed for a week and then used. The stimulating effect was reduced, but nitrogen fixation was still materially increased.

SUMMARY

A. chroococcum was grown in a synthetic medium to which various quantities of the following amino acids and proteins were added: glycine, leucine, dl-isoleucine, dl-valine, isovaline, l-aspartic acid, glutamic acid, dl-lysine, d-argi-

nine, dl-methionine, cystine, tyrosine, phenylalanine, l-tryptophane, hydroxyproline, l-histidine, l-proline, casein, albumin, and gelatin; and its nitrogen-fixing powers were determined.

Tyrosine, dl-isoleucine, hydroxyproline, and l-histidine greatly increased nitrogen fixation by *A. chroococcum*.

Phenylalanine and d-arginine were the only two amino acids used which materially retarded nitrogen fixation by *A. chroococcum*. The other amino acids tested either slightly increased fixation or were without effect.

Cystine and dl-methionine increased nitrogen fixation approximately 20 per cent, and their actions nearly paralleled each other throughout the concentrations tested.

Phenylalanine is the only amino acid tested which may occur in soils in sufficient concentrations to retard nitrogen fixation by *A. chroococcum*.

Casein and albumin materially increased nitrogen fixation by *Azotobacter*, whereas gelatin greatly retarded it.

REFERENCES

- (1) Association of Official Agricultural Chemists 1920 Official and Tentative Methods of Analysis, Washington, D. C.
- (2) BEAR, F. E. 1922 Nitrogen economy in soil. *Jour. Amer. Soc. Agron.* 14: 136-152.
- (3) BROWN, P. E., AND ALLISON, F. E. 1916 The influence of some common humus-forming materials of narrow and wide nitrogen-carbon ratio on bacterial action. *Soil Sci.* 1: 49-75.
- (4) BURK, D. AND LINEWEAVER, H. 1930 The influence of fixed nitrogen on *Azotobacter*. *Jour. Bact.* 19: 389-414.
- (5) COLEMAN, D. A. 1917 The influence of sodium nitrate upon nitrogen transformations in soils with special reference to its availability and that of other nitrogenous manures. *Soil Sci.* 4: 345-432.
- (6) DUGGELL, M. 1917 The importance of non-symbiotic nitrogen-fixing bacteria for plant nutrition. *Ortljschr. Naturf. Gessel. Zurich*, 62: 394-422. (Abs. in *Exp. Sta. Rec.* 42: 18.)
- (7) DVORAK, J. 1912 Studien ueber die Stickstoffanhaufung im Boden durch Microorganismen. *Ztschr. Landw. Versuchsw. Oesterr.* 15: 1077-1121.
- (8) FULLER, J. E., AND RETTGER, L. F. 1931 The influence of combined nitrogen on growth and nitrogen fixation by *Azotobacter*. *Soil Sci.* 31: 219-234.
- (9) FULMER, H. L., AND FRED, E. B. 1917 Nitrogen-assimilating organisms in manure. *Jour. Bact.* 2: 422-434.
- (10) GREAVES, J. E. 1933 Some factors influencing nitrogen fixation. *Soil Sci.* 36: 267-280.
- (11) GREAVES, J. E., AND ANDERSON, A. 1936 Sulfur requirements of *Azotobacter chroococcum*. *Soil Sci.* 41: 197-201.
- (12) GREAVES, J. E., AND BRACKEN, A. F. 1939 The influence of cropping on the nitrogen-fixing powers of soil. *Soil Sci.* 47: 201-206.
- (13) GREAVES, J. E., AND NELSON, D. H. 1923 The influence of nitrogen in soil on azo-fixation. *Utah Agr. Exp. Sta. Bul.* 185.
- (14) HANZAWA, J. 1914 Einige Beobachtungen über Stickstoffbindung durch *Azotobacter* in stickstoffarmen und in stickstoffreichen Substraten. *Centbl. Bakt.* (II) 41: 573-576.
- (15) HEADDEN, W. P. 1912 Deterioration in the quality of sugar beets due to the nitrates formed in the soil. *Colo. Agr. Exp. Sta. Bul.* 183.

- (16) HEINZE, B. 1910 Bodenbakteriologische Untersuchungen. *Landw. Jahrb.* 39 (Ergänzungsband 3): 515-543.
- (17) HILLS, T. L. 1918 Influence of nitrates on nitrogen-assimilating bacteria. *Jour. Agr. Res.* 12: 183-230.
- (18) HILTNER, L., AND STORMER, K. 1903 Studien ueber die Bacterienflora des Ackerbodens, etc. Paul Parey, Berlin.
- (19) HUTCHINSON, H. B. 1918 The influence of plant residues on nitrogen fixation and on losses of nitrate in the soil. *Jour. Agr. Sci.* 9: 92-111.
- (20) JODIDI, S. L. 1910 Organic nitrogenous compounds in peat soils. *Amer. Chem. Soc. Jour.* 32: 396-410.
- (21) JODIDI, S. L. 1911 The chemical nature of the organic nitrogen in soil. Iowa Agr. Exp. Sta. Res. Bul. 142.
- (22) KOSTYTSCHEW, S., RYSKALITCHUK, A., AND SCHWEZOWA, O. 1926 Biochemische Untersuchungen über *Azotobacter agile*. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 154: 1-17.
- (23) KRAINSKI, A. V. 1912 Enriching of soil in nitrogen in connection with the life activity of aerobic microorganisms assimilating free-nitrogen. *Univ. Izv. (Kief)* 42: (no. 4, pt. 2, art. 3) 1-58; (no. 8, pt. 2, art. 3) 59-151; (no. 9, pt. 2, art. 5) 133-182. (Abs. in *Exp. Sta. Rec.* 31: 721-722.)
- (24) LIPMAN, J. G. 1904 Experiments on the transformation and fixation of nitrogen by bacteria. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1903: 217-285.
- (25) LIPMAN, J. G. 1906 *Azotobacter* studies. *N. J. Agr. Exp. Sta. Ann. Rpt.* 1905: 254-280.
- (26) LÖHNIS, F., AND GREEN, H. H. 1914 Über die Entstehung und die Zersetzung von Humus, sowie über dessen Einwirkung auf die Stickstoff-Assimilation. *Centbl. Bakt. (II)* 40: 52-60.
- (27) MCBETH, I. G. 1913 Cellulose as a source of energy for nitrogen fixation. U. S. Dept. Agr. Bur. Plant Indus. Cir. 131.
- (28) MOCKERIDGE, F. A. 1912 Some conditions influencing the growth of *Azotobacter* and the fixation of nitrogen by the organism. *Ann. Bot.* 26: 871-887.
- (29) MURRAY, T. J. 1917 The effect of different plant tissues on the fixation of atmospheric nitrogen. *Va. Agr. Exp. Sta. Tech. Bul.* 15: 93-102.
- (30) REED, H. S., AND WILLIAMS, B. 1917 The effect of some organic soil constituents upon nitrogen fixation by *Azotobacter*. *Va. Agr. Exp. Sta. Tech. Bul.* 4: 81-95.
- (31) RICHARDS, E. H. 1917 The fixation of nitrogen in feces. *Jour. Agr. Sci.* 8: 299-311.
- (32) RIGOTARD, M. 1935 Note on the amino nitrogen in some soils of warm regions. *Trans. Third. Internatl. Cong. Soil Sci.* 1: 108-109.
- (33) STOKLASA, J. 1908 Beitrag zur Kenntnis der chemischen Vorgänge bei der Assimilation des elementaren Stickstoffs durch *Azotobacter* und *Radiobacter*. *Centbl. Bakt. (II)* 21: 484-509; 620-632.
- (34) STRANAK, FR. 1909 Zur Assimilation des Luftstickstoffes durch im freilebende Microorganismen. *Ztschr. Zuckerindus. Böhmen* 33: 599-614. (Abs. in *Chem. Abs.* 3: 2192.)
- (35) THOMPSON, L. G., JR. 1932 Nitrogen changes produced in certain nitrogenous compounds by *Azotobacter* and the nitrogen fixed in the presence of these compounds. *Jour. Agr. Res.* 45: 149-161.
- (36) WINTERS, N. E. 1924 Soil conditions which promote nitrogen fixation. *Jour. Amer. Soc. Agron.* 16: 701-716.
- (37) ZOOND, A. 1926 The relation of combined nitrogen to the physiological activity of *Azotobacter*. *Brit. Jour. Exp. Biol.* 4: 105-113.

SURVIVAL OF AZOTOBACTER IN SOIL¹

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Azotobacter is a widely distributed typical soil organism: approximately half of the soils examined throughout the world were found to contain this organism (12). The presence of Azotobacter or its survival in the soil after it has been introduced is apparently contingent upon the presence of available carbohydrates or other energy sources; the presence of minerals, such as calcium, phosphate, potassium, iron, and molybdenum; proper soil aeration; suitable temperature and moisture; and a favorable soil reaction of pH 6.0 and above (11, 35).

Liming was found to produce a favorable effect upon the development of Azotobacter, especially in soils of humid regions (2, 12, 14, 15, 31). Wilson and Wilson (36) found, however, that CaCO_3 exerted an inhibiting effect on Azotobacter. They suggested that the carbonate-phosphate ratio might affect the development of the organism, particularly since the application of K_2HPO_4 to limed soil resulted in multiplication of Azotobacter. These results were not confirmed (23). Magnesium carbonate can replace CaCO_3 and may give even greater nitrogen fixation (3, 14).

The addition of potassium salts and of phosphates to soils has frequently resulted in the stimulation of Azotobacter activity (1, 15, 23, 25, 31, 36), with certain exceptions (22, 32, 41). Molybdenum is essential to nitrogen fixation but is not essential to growth, since it is not effective in the presence of fixed nitrogen (4, 5, 6). Steinberg (30) reported, however, that molybdenum is essential for growth, even in the presence of fixed nitrogen. Van Niel (34) suggested the use of Azotobacter, by means of the soil plaque method, for determining molybdenum deficiency in soils.

The addition to soils of various organic materials, such as green manure and fresh straw, has been found to enhance nitrogen fixation (35). It has been suggested that these residues serve, aside from their physical effect upon the soils, as potential sources of energy for the nitrogen-fixing bacteria. Other investigators (18, 24) claimed that this effect is due to the growth-promoting substances in the organic residues. It has also been suggested that these complex plant constituents protect Azotobacter against poisons or toxins. The presence of iron, aluminum, silicon, and other elements in the soil humus

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is believed to be largely responsible for the observed increases in nitrogen fixation (9, 19, 20, 23) in culture media containing soil extracts.

It has long been recognized that fixed nitrogen, in the form of nitrate or ammonia, inhibits nitrogen fixation, since *Azotobacter* utilizes these compounds for growth in preference to the free nitrogen (6, 8, 17). Winters (40) found that small applications of available nitrogen to soil stimulated nitrogen fixation, whereas larger amounts repressed it. A concentration of 0.8–1 per cent of $(\text{NH}_4)_2\text{SO}_4$ was found (27) to be optimum for *Azotobacter* development, but larger amounts were definitely inhibitory. Ziemiecka (42) and Winogradsky (38) concluded that, in fertile soils containing an abundance of nitrogen, *Azotobacter* may be depressed and, in many instances, entirely eliminated, since this organism cannot effectively compete with the rest of the soil population. Lochhead (21, p. 5–39) also observed a depressing effect of nitrate fertilization on *Azotobacter*, an observation not supported by Martin and Brown (22).

In the following experiments an attempt was made to study the factors concerned in the establishment of *Azotobacter* in soils.

EXPERIMENTAL

Palouse silt loam² supported at one time an active *Azotobacter* flora (32). In recent years, however, the *Azotobacter* population in this soil has decreased to the point where it can no longer be demonstrated. This soil and four soils obtained from the New Jersey Agricultural Experiment Station plots were used in the following experiments. The treatments and composition of the soils are as follows (16, 33):

SOIL	TREATMENT	pH	ORGANIC C <i>per cent</i>	TOTAL N <i>per cent</i>
Palouse silt loam Sassafras loam	Untreated virgin soil, in native grass	6.2	3.46	0.209
Plot 7A	Untreated	4.3	0.61	0.066
Plot 7B	Limed (2 tons per acre) every 5 years	6.3	1.02	0.080
Plot 5A	Unlimed, minerals*, cow manure	5.1	1.80	0.155
Plot 5B	Limed, minerals*, cow manure	6.4	1.90	0.145

* Minerals: 320 pounds of superphosphate, 160 pounds muriate of potash, 320 pounds nitrate of soda per acre; cow manure: 16 tons per acre.

The soils were air-dried and passed through a 2-mm. sieve. Three-hundred-gram portions were placed in glass jars, brought to 50 per cent of the moisture-holding capacity, and inoculated with a suspension of *Azotobacter chroococcum* prepared from 48-hour mannite-agar cultures. The soils were allowed to stand for 24 hours so as to permit a uniform distribution of the moisture and organisms; samples were then removed for plating. All jars were weighed,

² The author is indebted to S. C. Vandecaveye, of the State College of Washington, Pullman, Wash., for supplying this soil.

covered, and incubated at 28°C.; moisture was maintained at the optimum by weekly additions of water.

The procedure of plating for *Azotobacter* was, in essence, a modification of Winogradsky's silica-gel technic (37). The soil was removed from the glass jars and spread in a thin layer on paper until air dry (about 2 hours). It was then finely powdered with a spatula. Various quantities were weighed out accurately and spread as evenly as possible over the surface of sterile nitrogen-free mannite agar placed in 15-cm. Petri plates. Counts were made of *Azotobacter* colonies after 4-5 days' incubation at 28°C. The method is simple and rapid, although only semiquantitative. Table 1 presents counts of *Azotobacter* made in a preliminary study of the untreated soils. The uninoculated soils contained no *Azotobacter*, as determined by the foregoing method.

A marked decrease in the numbers of *Az. chroococcum* occurred in every soil but especially in soils 7A and 5A; it was seldom possible to recover the organ-

TABLE 1
Survival of Azotobacter in untreated soils
Numbers per gram of oven-dry soil

SOIL USED	DAYS OF INCUBATION				
	0	20	45	75	100
Palouse silt loam . . .	480	130	100	50	25
Soil 7A	0	0, 0*	0, 0*	0, 0*	0, 0*
Soil 7B	285	140	0	0	0
Soil 5A	200	0	0, 300*	0, 250*	0
Soil 5B	360	250	120	25	0

* Reinoculated.

ism 24 hours after its inoculation into these two soils. It seems to be well established (7, 10, 11) that reactions lower than pH 6.0 are destructive to this organism.

The pH factor does not explain the elimination of *Azotobacter* from soils 7B, 5B, and Palouse silt loam, since it was found that the strain of *Az. chroococcum* could develop at pH 6.0 in artificial media.

These soils were next treated with various organic and inorganic materials in order to determine the factors influencing the survival of *Azotobacter* in soil. In all cases 300-gm. portions of soil were used. The soluble materials were added together with the suspension of *Azotobacter* as the inoculum; lime, mannite, and the organic residues were thoroughly mixed with the dry soil prior to inoculation. The results are presented in table 2.

The addition of lime and molybdenum, alone and together, favored the persistence of *Azotobacter* in soil; CaCO_3 seems to have exerted a slightly stimulating effect upon this organism. Because of the inaccuracy of the plat-

ing method, however, such differences need not be significant. The reaction alone was evidently not the factor responsible for the disappearance of the organism in the soil, as the pH values for the control and for the molybdenum-treated soils were about the same.

Soils treated with alfalfa, straw, and manure did not show any differences from those receiving CaCO_3 and molybdenum. The conclusion may, there-

TABLE 2
Survival of Az. chroococcum in Palouse soil, differently treated
Numbers per gram of oven-dry soil

SOIL TREATMENT*	DAYS OF INCUBATION					FINAL pH
	0	26	63	130	217	
1. Control.....	1,300	1,200	210	0	0	6.1
2. CaCO_3	1,310	5,000	9,000	2,000	3,500	7.3
3. Na_2MoO_4	1,250	1,100	3,200	2,200	2,200	6.2
4. $\text{CaCO}_3 + \text{Na}_2\text{MoO}_4$	1,450	7,580	4,620	3,400	5,400	7.0
5. Alfalfa.....	1,340	3,160	2,300	5,000	3,720	7.1
6. Straw.....	1,200	3,000	4,500	4,400	4,200	7.2
7. Manure.....	1,150	3,920	2,800	2,800	3,600	6.9
8. Dried blood.....	1,190	0	0	0	0	6.6
9. Mannite.....	1,050	600,000	200,000	1,500,000	1,200,000	7.2
10. K_2HPO_4	1,390	240	640	20	5	7.8
11. Superphosphate.....	1,510	60	10	5	0	5.9
12. Alfalfa + K_2HPO_4	1,290	240	30	0	0	7.5
13. Straw + K_2HPO_4	1,530	700	600	140	0	7.7
14. Mannite + K_2HPO_4	1,190	580	340	100	100	7.6
15. $\text{K}_2\text{HPO}_4 + (\text{NH}_4)_2\text{SO}_4$..	1,460	75	105	0	0	6.1
16. Mannite + $\text{K}_2\text{HPO}_4 +$ $(\text{NH}_4)_2\text{SO}_4$	1,010	60	25	0	0	6.1
17. Straw + $\text{K}_2\text{HPO}_4 +$ $(\text{NH}_4)_2\text{SO}_4$	1,362	60	0	0	0	7.7
18. Superphosphate + $\text{K}_2\text{HPO}_4 + (\text{NH}_4)_2\text{SO}_4$	964	0	0	0	0	5.1
19. MgCO_3	1,280	1,300	4,200	5,000	3,800	7.6
20. $\text{MgCO}_3 + \text{Na}_2\text{MoO}_4$	1,450	3,000	6,400	2,800	2,900	7.5

* Treatments 3 and 4, Na_2MoO_4 added at rate of 0.01 per cent; all other materials applied at rate of 1 per cent; treatments 5 to 18 inclusive: 1 per cent $\text{CaCO}_3 + 0.01$ per cent Na_2MoO_4 .

fore, be drawn that the persistence of *Azotobacter* was due to the lime and molybdenum. The organic residues did not serve as sources of energy for this organism, within the experimental period.

Magnesium carbonate alone and with Na_2MoO_4 brought about conditions as favorable for *Azotobacter* as those with CaCO_3 and Na_2MoO_4 . This is in accord with the results of other investigators on the effect of magnesium (3, 8, 14). The addition of mannite resulted in a rapid increase in the number of *Azotobacter*.

Despite the presence of calcium and molybdenum, the remaining treatments brought about a marked decrease in the numbers of *Azotobacter*. Dried blood was definitely deleterious to the development of this organism. In the soils treated with dried blood, there were much greater numbers of microorganisms than in the soils treated with other organic residues; the competition for available nutrients, as well as the possible formation of toxic or lytic substances by the soil flora, may have been responsible for the rapid disappearance of the *Azotobacter* added to the soil (26).

It was surprising to note that the addition of both superphosphate and K_2HPO_4 brought about a pronounced decrease in the numbers of *Azotobacter*. The amounts added (1 per cent) were decidedly too high; yet the concentration of available phosphate, in the presence of $CaCO_3$, was probably somewhat low, since comparatively small amounts of phosphate would remain in solution

TABLE 3

*Influence of concentration of potassium phosphate on sugar consumption and nitrogen fixation by Az. chroococcum**

CONCENTRATION OF K_2HPO_4 AND KH_2PO_4 (9:1)	SUGAR CONSUMED	TOTAL NITROGEN
<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>
Uninoculated control	0.0	0.42
2.50	192.5	1.86
2.00	262.5	2.60
1.50	287.5	3.12
1.00	365.0	3.54
0.50	1190.0	15.09
0.10	1360.0	11.34
0.05	1360.0	11.87

* In nitrogen-free dextrose medium (dextrose, 1360 mgm.).

under such conditions. The presence of the phosphate, however, may have resulted in the precipitation of the iron or of other elements which the organism requires.

To test further the specific effect of potassium and phosphate, experiments were carried out with pure cultures of *Azotobacter* in a nitrogen-free dextrose medium containing varying amounts of a constant ratio of K_2HPO_4 and KH_2PO_4 (9:1); this buffer ratio was used to maintain the medium at a pH of 7.2. Comparatively little sugar was consumed with the higher concentrations of the salts; with the lower concentrations (0.5–0.05 per cent) the dextrose was rapidly destroyed. Nitrogen fixation was greatest with 0.5 per cent phosphate (table 3). These results show that concentrations of phosphate greater than 0.5 per cent suppressed the organism.

The soil inoculation experiments were repeated. As sources of energy, mannite and glucose were used, as well as two alcohols and calcium salts of certain aliphatic acids (13, 39). The results, reported in table 4, show that

Azotobacter developed rapidly with mannite and glucose alone and with CaCO_3 and Na_2MoO_4 , especially where the two inorganic constituents were added. The inhibitory effect of K_2HPO_4 was again apparent, although a decrease in numbers was not obtained where K_2HPO_4 alone was used. Of the other sources of energy, only calcium acetate enabled the organisms to survive. This effect may be due to the calcium itself rather than to the acetate, since the numbers of *Azotobacter* were no greater than with CaCO_3 alone (see table 2). The possibility suggested itself that the concentration of these energy sources was too great. A similar experiment was therefore carried out, using

TABLE 4
Influence of treatment on persistence of Az. chroococcum in Palouse soil
Numbers per gram of oven-dry soil

SOIL TREATMENT*	DAYS OF INCUBATION				FINAL pH
	0	12	40	108	
Control.....	1,000	880	450	50	6.4
Mannite.....	840	150,000	450,000	300,000	6.7
Mannite + CaCO_3 + Na_2MoO_4	1,240	206,000	900,000	4,000,000	7.6
K_2HPO_4	1,400	1,110	1,100	1,200	7.1
Mannite + CaCO_3 + Na_2MoO_4 + K_2HPO_4	1,360	2,000	650	45	7.7
Glucose.....	880	104,000	310,000	190,000	6.5
Glucose + CaCO_3 + Na_2MoO_4	1,310	138,000	320,000	900,000	7.4
Glucose + CaCO_3 + Na_2MoO_4 + K_2HPO_4	1,120	1,000	650	120	7.6
Calcium acetate.....	1,360	2,200	2,100	2,680	7.5
Calcium acetate + Na_2MoO_4 + CaCO_3 ..	1,110	1,900	3,900	3,420	7.4
Ethyl alcohol.....	850	0	0	0	6.3
Ethyl alcohol + CaCO_3 + Na_2MoO_4	930	30	0	0	7.4
Calcium benzoate.....	1,090	0	0	0	8.0
Calcium benzoate + CaCO_3 + Na_2 - MoO_4	1,330	35	15	4	7.7
Butyl alcohol.....	1,290	0	0	0	7.0
Butyl alcohol + CaCO_3 + Na_2MoO_4	880	0	0	0	7.7

* All materials added at rate of 1 per cent, except Na_2MoO_4 , 0.01 per cent.

one-half the concentration of all reagents except CaCO_3 and Na_2MoO_4 . The data given in table 5 indicate that the reduced concentration of the energy sources resulted in the persistence of *Azotobacter* in these soils. Ethyl alcohol and calcium acetate alone and especially in combination with calcium and molybdenum stimulated development of the nitrogen-fixing organisms.

An analysis of the numbers of fungi, bacteria, and actinomycetes in the experiment reported in table 4 revealed the fact that K_2HPO_4 alone had no marked effect on the fungi and actinomycetes but brought about some increase in the numbers of bacteria (fig. 1). The combination of the salt with mannite resulted in a striking increase in the numbers of fungi, bacteria, and actino-

mycetes. The possibility exists that the repression of *Azotobacter* by K_2HPO_4 was due to the successful competition by the soil microflora for nutrients and even to the production by this active population of substances toxic to *Azotobacter*. It is of interest, however, that *Azotobacter* increased rapidly in the presence of mannite or glucose, even though the other organisms were simultaneously multiplying rapidly. It is only where the unusually large numbers occurred as a result of the addition of K_2HPO_4 that inhibition of *Azotobacter*

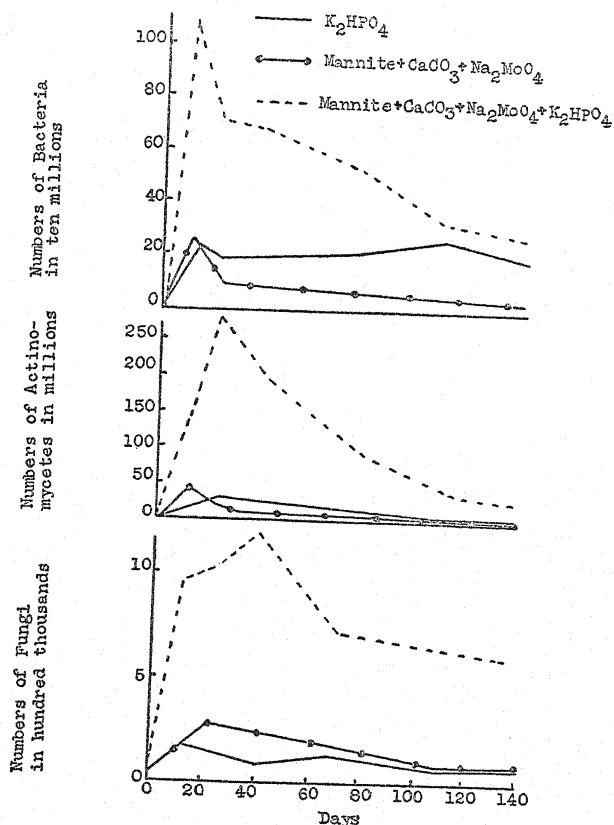


FIG. 1. NUMBERS OF FUNGI, ACTINOMYCETES, AND BACTERIA IN PALOUSE SOIL RECEIVING DIFFERENT TREATMENTS

was manifested. The development, in the presence of K_2HPO_4 , of a specific flora particularly antagonistic to *Azotobacter*, may also be inferred from these results.

It may be of interest to direct attention to the fact that Rybalkina (26) found that *Azotobacter chroococcum* failed to multiply and even died out when inoculated into peat. She concluded that this organism was inhibited or killed as a result of the toxicity of the peat itself, the presence of bacterial antagonists, or the devouring of large numbers by amoebae.

An attempt was next made to establish *Azotobacter* in soils 7A, 7B, 5A, and 5B. The results presented in table 6 show that in soil 7A only the addition of CaCO_3 favored the persistence of *Azotobacter*. In all other cases, this organism could not be demonstrated even 24 hours after it had been inoculated. The addition of readily available energy sources brought about an increase of *Azotobacter* where CaCO_3 and Na_2MoO_4 were present. The nitrogen-fixing organism persisted in 7B soils where lime was added; mannite and glucose, together with CaCO_3 , brought about an increase in its numbers.

The absence of lime in soil 5A was evidently responsible for the inability of *Azotobacter* to survive, although the organism could be recovered after inoculation. The pH of this soil is somewhat higher than that of soil 7A and, in addition, the organic matter in soil 5A may have exerted a partial though

TABLE 5
Persistence of Az. chroococcum in Palouse soil receiving different treatments
Numbers per gram oven-dry soil

SOIL TREATMENT*	DAYS OF INCUBATION				FINAL pH
	0	12	40	108	
Control.....	1,065	860	420	90	6.3
Mannite.....	1,110	45,000	80,000	150,000	6.3
Mannite + CaCO_3 + Na_2MoO_4 + K_2HPO_4 ..	990	1,190	1,360	1,200	7.4
Calcium acetate.....	1,460	1,580	1,660	2,600	7.5
Calcium acetate + CaCO_3 + Na_2MoO_4	1,360	2,100	2,900	3,600	7.7
Ethyl alcohol.....	1,210	2,600	4,300	5,000	6.5
Ethyl alcohol + CaCO_3 + Na_2MoO_4	1,290	2,650	5,000	7,540	7.3
Calcium benzoate.....	890	690	460	460	7.2
Calcium benzoate + CaCO_3 + Na_2MoO_4	960	1,010	864	965	7.6
Butyl alcohol.....	1,300	1,190	720	250	6.9
Butyl alcohol + CaCO_3 + Na_2MoO_4	1,190	1,220	650	310	7.3

* CaCO_3 and Na_2MoO_4 added at rate of 1 per cent and 0.01 per cent respectively, all other treatments 0.5 per cent.

short-lived protective action against whatever factor was responsible for the disappearance of the organisms. This property has been ascribed to organic matter by various investigators (23, 35).

The reaction of soil 5B was evidently not harmful to *Az. chroococcum*, since this organism persisted even in the absence of lime. Nevertheless, CaCO_3 was necessary for an increase of *Azotobacter* in the presence of mannite or glucose. The addition of mannite, lime, and molybdenum to the uninoculated soil, did not result in any development of *Azotobacter*.

It thus appears that soils show marked differences in regard to their ability to support *Azotobacter* even in the presence of lime, molybdenum, and readily available energy sources. Other factors to be considered are the presence of phosphorus, iron, and aluminum; the physical condition of the soil; and the presence of organisms antagonistic to *Azotobacter*.

The extraordinarily rapid disappearance of *Az. chroococcum* in soil 7A was so striking that attempts were made to obtain some evidence as to the responsible factors. An acid-tolerant nitrogen-fixing bacterium, *Az. indicum*, isolated by Starkey and De (28, 29), was next used as the test organism. Platings were made as before except that dextrose was used as the energy source (29). Accurate counts could be made only with difficulty, since the organisms developed very slowly (7-10 days) on the plates; these tended, in the meantime, to be overrun by various organisms, including a rapidly growing yeast. Nevertheless, the counts, even though only approximate, are of interest (table 7).

TABLE 6

Persistence of Az. chroococcum in soils 7A, 7B, 5A, and 5B treated with various materials
Numbers of organisms per gram oven-dry soil, $\times 10^{-2}$

DAYS OF INCUBATION.....	SOIL 7A				SOIL 7B				SOIL 5A				SOIL 5B			
	0	12	40	105	0	12	40	105	0	12	40	105	0	12	40	105
<i>Soil Treatment*</i>																
Control.....	0	0	0	0	35	0	0	0	15	0	0	0	18	10	7	1
CaCO ₃	23	24	21	29	22	35	29	24	10	15	19	22	20	35	40	31
Na ₂ MoO ₄	0	0	0	0	36	9	5	6	19	0	0	0	27	18	21	25
CaCO ₃ + Na ₂ MoO ₄	19	23	26	21	29	21	22	40	11	14	25	19	16	32	35	100
Alfalfa.....	0	0	0	0	27	8	6	7	15	0	0	0	30	15	18	11
Straw.....	0	0	0	0	21	10	5	5	21	0	0	0	19	22	10	12
Manure.....	0	0	0	0	25	12	6	9	9	0	0	0	25	21	18	14
Mannite.....	0	0	0	0	28	24	27	40	16	0	0	0	21	29	42	47
Glucose.....	0	0	0	0	32	34	40	45	18	0	0	0	27	22	35	36
Alfalfa + CaCO ₃ + Na ₂ - MoO ₄	24	19	18	16	39	25	21	23	13	9	15	10	20	35	41	35
Straw + CaCO ₃ + Na ₂ - MoO ₄	18	17	31	18	29	26	29	22	22	18	12	15	29	31	36	29
Manure + CaCO ₃ + Na ₂ - MoO ₄	22	20	26	20	21	27	35	21	11	8	13	9	21	18	29	25
Mannite + CaCO ₃ + Na ₂ - MoO ₄	16	24	35	100	26	95	156	460	10	15	36	155	18	90	180	720
Glucose + CaCO ₃ + Na ₂ - MoO ₄	19	16	26	85	35	60	121	350	18	12	30	128	28	80	150	600

* Na₂MoO₄ added at rate of 0.01 per cent, remaining treatments 1 per cent.

The unlimed soil of plot 7A completely inhibited or destroyed *Az. indicum*, tolerant as it is in pure culture to a pH as low as 3.0 (28). The beneficial effect of lime was apparent, but the organism decreased, in time, with lime alone. This decrease may have been due to lack of an available energy source, for when dextrose was supplied, as in treatments 7 and 8, the organism actually multiplied.

A soil as acid as soil 7A undoubtedly contains dissolved iron and aluminum (16). An experiment was therefore arranged to test the effect of soluble aluminum, as Al₂(SO₄)₃, at different pH levels on both *Az. chroococcum* and

Az. indicum. Nitrogen-free dextrose medium was placed in 250-cc. Erlenmeyer flasks and inoculated with uniform suspensions of each organism. Tests for the survival of the organisms were made by spreading 1-cc. portions of the inoculated media on the surface of nitrogen-free mannitol and dextrose agars in Petri plates. It is shown in table 8 that *Azotobacter chroococcum* cannot withstand a pH of 4.0 for 24 hours or a pH of 5.0 for 4 days; the latter reaction also restricted development of the organism in 24 hours. The in-

TABLE 7
Survival of *Az. indicum* in soil 7A
Numbers per gram of oven-dry soil

INCUBATION	UNLIMED		LIMED*		UNLIMED + DEXTROSE*		LIMED + DEXTROSE*	
	1	2	3	4	5	6	7	8
days								
0†	0	0	8,400	8,800	0	0	9,000	8,400
13	0	0	2,320	2,900	0	0	28,000	21,000
25	0	0	1,500	1,200	0	0	35,000	29,000
pH.....	4.1	4.2	7.0	7.1	4.3	4.1	6.9	7.1

* Lime and dextrose, where used, were added at the rate of 1 per cent.

† Zero plating 24 hours after inoculation.

TABLE 8
Influence of reaction and of aluminum on the survival of *Az. chroococcum* in liquid media*

REACTION.....pH	4.0				5.0				6.0				7.0			
Days of incubation....	0	1	4	10	0	1	4	10	0	1	4	10	0	1	4	10
<i>Al</i> (SO ₄) ₂ p.p.m.																
0	4	0	0	0	4	2	0	0	4	4	4	2	4	4	4	3
10	4	0	0	0	4	0	0	0	4	3	2	2	4	4	4	3
25	4	0	0	0	4	0	0	0	4	3	2	1	4	3	4	2
50	4	0	0	0	4	0	0	0	4	3	2	0	4	3	3	2
75	4	0	0	0	4	0	0	0	4	2	1	0	4	3	3	2
100	4	0	0	0	4	0	0	0	4	2	0	0	4	3	3	2

* 0 = no growth, 1 = slight, 2 = fair, 3 = medium, 4 = abundant.

hibitive effect of aluminum was clearly apparent, 10 p.p.m. at pH 5.0 being sufficient to inactivate *Azotobacter* in 24 hours. At pH 6.0 aluminum again exerted a suppressing influence in 24 hours at concentrations of 75 to 100 p.p.m. and at a concentration of 10 p.p.m. in 4 days. A toxic effect of this element was noted even at pH 7.0, although at this high reaction the effect was less pronounced because some of the aluminum was probably precipitated.

The data given in table 9 indicate that *Az. indicum* was much more tolerant than *Az. chroococcum* to high acidity even with relatively high concentrations

of aluminum. By the tenth day, however, there was some evidence of repression at pH 4.0 with 100 p.p.m. of $\text{Al}_2(\text{SO}_4)_3$. Comparison of turbidities of the different culture flasks indicates that there was definite inhibition with increasing concentrations of aluminum. This was particularly pronounced at pH 4.0 and was noticeable also at pH 5.0 with 75 and 100 p.p.m. of the aluminum salt.

It might be argued that the sulfate radical was the toxic one, but as is shown in the following experiment, both the organisms used in this study were tolerant to much greater concentrations of sulfate than those used in the foregoing experiment.

In order to prove whether toxic substances are present in soil 7A, a quantity of this soil was extracted with distilled water for 2 hours with constant shaking. After being passed through paper, the filtrate was divided into six 30-cc. por-

TABLE 9

*Influence of reaction and of aluminum on the survival of Az. indicum on agar and in liquid media**

REACTION..... <i>pH</i>	4.0				5.0				6.0				7.0			
Concentration of Al ₂ (SO ₄) ₃ <i>p.p.m.</i>	0	10	50	100	0	10	50	100	0	10	50	100	0	10	50	100
<i>days</i>																
<i>Growth on agar</i>																
0	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
1	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
4	3	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
10	3	3	3	2	4	4	4	4	4	4	4	4	4	4	4	4
<i>Growth in liquid media, relative turbidity</i>																
10	3	2	2	1	4	4	4	2	4	4	4	4	4	4	4	4
20	3	2	2	1	4	4	4	3	4	4	4	3	4	4	4	4

* 1 = slight growth, 2 = fair, 3 = medium, 4 = abundant.

tions. Two were filtered through sterile Berkfeld (N) candles, two were sterilized at 15 pounds for 15 minutes, and two were evaporated to dryness and ashed. The ash was taken up with concentrated sulfuric acid and brought to pH 6.7 with NaOH; the volume of each sample was made to 30 cc., and both aliquots were sterilized for 15 minutes at 15 pounds; the liquids contained an abundance of flocculent aluminum and iron. One set of these preparations was inoculated with *Az. chroococcum*, and the other with *Az. indicum*. Tests were made immediately after inoculation and at various intervals.

The data presented in table 10 indicate that *Az. indicum* can survive for at least 10 days in the soil filtrates at pH 4.4, which contained considerable soluble iron and aluminum, and in the solution of the ashed filtrate adjusted to pH 6.7. *Az. chroococcum* was much less tolerant: the cells were completely destroyed in the acid filtrate in 24 hours, and the nearly neutral liquid was also

somewhat toxic. The reaction of the Berkfeld and sterilized filtrates inoculated with *Az. chroococcum* was adjusted to pH 7.0, and a heavy precipitate of iron and aluminum was formed. The filtrates were once more inoculated with *Azotobacter chroococcum*, and the organism could be demonstrated even after 4 days. The combined effects of reaction, iron, and aluminum seem, therefore, to be responsible for the inhibition of this organism at pH 4.4.

The foregoing studies suggest that a low pH and possibly soluble aluminum are the factors chiefly responsible for the suppression of *Az. chroococcum* in soil 7A. The reason for the inhibition of *Az. indicum* in the same soil, untreated, is still unknown.

TABLE 10
*Influence of soil extract upon the development of Azotobacter**

INCUBATION	<i>Az. chroococcum</i>			<i>Az. indicum</i>		
	Berkfeld filtrate	Sterilized filtrate	Ashed filtrate	Berkfeld filtrate	Sterilized filtrate	Ashed filtrate
days						
0	4	4	4	4	4	4
1	0	0	2	4	4	4
4	0	0	1	4	4	4
10	0	0	1	4	4	4
pH.	4.4	4.4	6.7	4.4	4.4	6.7

* 0 = no growth, 1 = slight, 2 = fair, 4 = abundant.

SUMMARY

Five soils, Palouse silt loam and four soils from the New Jersey Agricultural Experiment Station plots 7A, 7B, 5A, and 5B, were found to lack *Azotobacter*. Attempts were made to determine the factors which may lead to the establishment of this organism in these soils.

The inoculation of *Az. chroococcum* resulted in a sharp decrease in numbers, especially in the more acid soils 7A and 5A, where they tended to disappear shortly after introduction. No lytic agents could be isolated from these soils.

The inoculation of *Az. chroococcum* into the Palouse soil receiving different treatments brought out several interesting facts. The organism survived when molybdenum and CaCO_3 were added alone or together. An increase in numbers occurred only in the presence of readily available sources of energy, such as mannite or glucose, as well as with ethyl alcohol and calcium acetate in appropriate concentrations (0.5 per cent); at higher concentrations (1 per cent), ethyl alcohol, butyl alcohol, and calcium benzoate completely suppressed *Azotobacter*. Alfalfa, straw, and manure did not stimulate the development of *Azotobacter*, nor did they prove harmful. Dried blood was definitely injurious. Phosphate, alone or in combination with various organic materials

(mannitol, glucose, straw, etc.) was inhibitive in concentrations of 1 per cent and 0.5 per cent. Pure culture studies indicated that a 1 per cent concentration of K_2HPO_4 and KH_2PO_4 , in the ratio of 9:1, markedly inhibited sugar consumption and nitrogen fixation. Counts of soil microorganisms were significantly higher in the presence of K_2HPO_4 , alone and particularly when combined with glucose or mannitol, than those of the same soils with carbohydrates alone. The unsuccessful competition with this microflora for nutrients was suggested as a probable explanation for the inhibition of Azotobacter in phosphate-treated soils.

Az. chroococcum was inoculated into soils from the station experimental plots 5A, 5B, 7A, and 7B, which were modified by various treatments. This organism persisted in soils 5A and 7A only upon addition of lime. The addition of readily available sources of energy to these soils, in the presence of lime, stimulated multiplication of Azotobacter to a certain extent. It was possible to recover the organism from the limed soils 7B and 5B even after a considerable period had elapsed after inoculation; mannite or glucose enabled the organism to develop in the presence of $CaCO_3$.

Different soils were shown to vary in their ability to support Azotobacter, even though the reaction was favorable and readily available sources of energy were supplied. Unsuccessful competition with the microflora and microfauna of these soils, the presence of toxic substances, and the absence of certain nutrients, such as phosphate and potassium, may be considered as among the factors responsible for the inability of Azotobacter to survive in these soils.

Az. indicum, an acid-tolerant nitrogen-fixing organism, could not survive in the acid soil 7A for 24 hours, but in the presence of lime it persisted, and upon addition of lime and dextrose it multiplied. Heavy suspensions of this organism as well as of *Az. chroococcum* were added to nitrogen-free dextrose media, adjusted to different pH levels and containing varying amounts of $Al_2(SO_4)_3$. The latter organism could not persist for 24 hours at pH 4.0 and decreased rapidly at pH 5.0. A low pH (4.0) and a low aluminum content did not appreciably affect *Az. indicum*, but higher concentrations of aluminum sulfate (75 to 100 p.p.m.), especially at pH 4.0, definitely delayed development though they did not completely stop its growth. It was concluded that both the low pH and the soluble aluminum were partly responsible for the disappearance of *Az. chroococcum* in the acid soil 7A, but no explanation could be found for the elimination of *Az. indicum* from this soil in 24 hours. Water extracts of this soil yielded no clue as to the inhibiting factor. The low pH of these extracts (pH 4.4) was sufficient to inhibit *Az. chroococcum* but not *Az. indicum*. Though this extract contained considerable soluble iron and aluminum, it was not inhibitive to either organism when the reaction was adjusted to pH 6.7-7.0 or to *Az. indicum* at pH 4.4. Whatever the destructive factor was, the presence of lime was sufficient to overcome its deleterious effects.

The experiments indicate that it is possible, by appropriate soil amendments, to establish *Azotobacter* and to stimulate its development in soils originally inimical to it.

REFERENCES

- (1) AQUINO, D. I. 1931 A non-symbiotic nitrogen-fixing organism of the genus *Azotobacter* in some Philippine soils. *Philippine Agr.* 20: 187-194.
- (2) AQUINO, D. I., AND VILLEGAS, L. J. 1932 A study of the occurrence of *Azotobacter* flora in some Philippine soils. *Philippine Agr.* 21: 695-706.
- (3) ASHEY, S. F. 1907 Some observations on the assimilation of atmospheric nitrogen by a free living soil organism. *Jour. Agr. Sci.* 11: 35-51.
- (4) BORTELS, H. 1930 Molybdän als Katalysator bei der biologischen Stickstoffbindung. *Arch. Mikrobiol.* 1: 333-342.
- (5) BORTELS, H. 1936 Weitere Untersuchungen über die Bedeutung von Molybdän, Vanadium, Wolfram und anderen Erdaschenstoffen für Stickstoffbindende und andere Mikroorganismen. *Centbl. Bakt.* (II) 95: 193-218.
- (6) BURK, D., AND HORNER, C. K. 1935 The specific catalytic role of molybdenum and vanadium in nitrogen fixation and amide utilization by *Azotobacter*. *Trans. Third Internat. Cong. Soil Sci.* 1: 152-155.
- (7) BURK, D., HORNER, C. K., AND LINEWEAVER, H. 1932 Injury and recovery of respiration and catalase activity in *Azotobacter*. *Jour. Cell. and Compar. Physiol.* 1: 435-449.
- (8) BURK, D., AND LINEWEAVER, H. 1930 The influence of fixed nitrogen on *Azotobacter*. *Jour. Bact.* 19: 389-414.
- (9) BURK, D., LINEWEAVER, H., AND HORNER, C. K. 1932 Iron in relation to the stimulation of growth by humic acid. *Soil Sci.* 33: 413-453.
- (10) BURK, D., LINEWEAVER, H., AND HORNER, C. K. 1934 The specific influence of acidity on the mechanism of nitrogen fixation by *Azotobacter*. *Jour. Bact.* 27: 325-340.
- (11) GAINNEY, P. L. 1918 Soil reaction and the growth of *Azotobacter*. *Jour. Agr. Res.* 14: 265-271.
- (12) GAINNEY, P. L. 1927 The occurrence of *Azotobacter* in soil. *Proc. First Internat. Cong. Soil Sci.* 4: 1-11.
- (13) GAINNEY, P. L. 1928 Sources of energy for *Azotobacter* with special reference to fatty acids. *Ann. Missouri Bot. Gard.* 15: 113-168.
- (14) GAINNEY, P. L. 1930 A study of factors influencing inoculation experiments with *Azotobacter*. *Kans. Agr. Exp. Sta. Tech. Bul.* 26: 3-66.
- (15) GERLACH, M. 1934 Zur Stickstoffsammlung in Ackerboden. *Landw. Jahrb.* 80: 73-101.
- (16) GOSS, D. M., AND PRINCE, A. L. 1935 Results of rapid tests applied to soils of known fertilizer treatment. *Amer. Soc. Agron. Com. on Fert. Proc.* 1: 94-101.
- (17) HILLS, T. L. 1918 The influence of nitrate on nitrogen assimilating bacteria. *Jour. Agr. Res.* 12: 183-230.
- (18) ITANO, A. 1923 Physiological study of *Azotobacter chroococcum*: I. Influence of vitamin B and nucleic acids on *Azotobacter*. *Jour. Bact.* 8: 483-486.
- (19) KASERER, H. 1910 Zur Kenntnis des Mineralstoffbedarfs von *Azotobacter*. *Ber. Deut. Bot. Gesell.* 28: 208-262.
- (20) KASERER, H. 1912 Über die biologische Reizwirkung natürlicher Humusstoffe. *Centbl. Bakt.* (II) 31: 577-578.
- (21) LOCHHEAD, A. G. 1938 Progress Report of the Dominion Agricultural Bacteriologist for the years 1934, 1935, 1936. Div. Bact., Dominion Exp. Farms, Dept. Agr. Ottawa, Ont., Canada.

- (22) MARTIN, W. P., AND BROWN, P. E. 1938 Factors influencing the occurrence of *Azotobacter* in Iowa soils. *Soil Sci.* 45: 455-466.
- (23) MARTIN, W. P., WALKER, R. H., AND BROWN, P. E. 1937 The occurrence of *Azotobacter* in Iowa soils and factors affecting their distribution. *Iowa Agr. Exp. Sta. Res. Bul.* 217.
- (24) MOCKERIDGE, F. A. 1915 Some effects of organic growth promoting substances (auximones) on the soil organisms concerned in the nitrogen cycle. *Proc. Roy. Soc. [London]* B. 89: 508-533.
- (25) NOVOGRUDSKY, D., AND NAUMOVA, K. 1932 Causes of "inactivity" of *Azotobacter*. *Microbiol. (U. S. S. R.)* 1: 181-191. (German Summary.)
- (26) RYBALKINA, A. V. 1938 The viability of the culture *Az. chroococcum* (Beijer.) in peat. *Microbiol. (U. S. S. R.)* 7: 935. (English summary.)
- (27) SCHWARTZ, W., AND MÜLLER, W. 1931 Einwirkung von künstlichen Düngen, besonders von Ammonsulfat auf Boden Organismen. *Arch. Mikrobiol.* 2: 621-638.
- (28) STARKEY, R. L. 1939 A species of *Azotobacter* tolerant to high acidity. *Science* 24: 267.
- (29) STARKEY, R. L., AND DE, R. K. 1939 A new species of *Azotobacter*. *Soil Sci.* 47: 329-343.
- (30) STEINBERG, R. A. 1938. Applicability of nutrient-solution purification to the study of trace-element requirements of *Rhizobium* and *Azotobacter*. *Jour. Agr. Res.* 57: 461-476.
- (31) TURK, L. M. 1935 Studies of nitrogen fixation in some Michigan soils. *Mich. Agr. Expt. Sta. Tech. Bul.* 143: 1-36.
- (32) VANDECAVEYE, S. C., AND ANDERSON, S. 1934 Longevity of *Azotobacter* in soils treated with lime and superphosphate. *Jour. Amer. Soc. Agron.* 26: 353-363.
- (33) VANDECAVEYE, S. C., AND KATZNELSON, H. 1938 Microbial activities in soil: V. Microbial activity and organic matter transformation in Palouse and Helmer soils. *Soil Sci.* 46: 139-167.
- (34) VAN NIEL, C. B. 1935 A note on the apparent absence of *Azotobacter* in soils. *Arch. Mikrobiol.* 6: 215-218.
- (35) WAKSMAN, S. A. 1932 Principles of Soil Microbiology, ed. 2. Williams & Wilkins Co., Baltimore.
- (36) WILSON, J. K., AND WILSON, B. D. 1933 The occurrence of *Azotobacter* in peat soils of New York. N. Y. (Cornell) Agr. Exp. Sta. Mem. 148.
- (37) WINOGRADSKY, S. 1925 Sur une méthode pour apprécier le pouvoir fixateur de l'azote dans les terres. *Compt. Rend. Acad. Sci. [Paris]* 180: 711-716.
- (38) WINOGRADSKY, S. 1935 The method in soil microbiology as illustrated by studies on *Azotobacter* and the nitrifying bacteria. *Soil Sci.* 40: 59-77.
- (39) WINOGRADSKY, S. 1938 Études sur la microbiologie du sol et des eaux. Sur la morphologie et l'écologie des *Azotobacter*. *Ann. Inst. Pasteur* 60: 1-50.
- (40) WINTERS, N. W. 1924 Soil conditions which promote nitrogen fixation. *Jour. Amer. Soc. Agron.* 16: 701-706.
- (41) ZIEMIECKA, J. 1930 Microbiological tests of the soil's fertility, nitrification and nitrogen fixation. *Proc. Second Internat. Cong. Soil Sci.* 3: 53-55.
- (42) ZIEMIECKA, J. 1932 The *Azotobacter* test of soil fertility applied to the classical fields at Rothamsted. *Jour. Agr. Sci.* 22: 797-810.

EFFECT OF WASTE SULFITE LIQUOR ON SOIL PROPERTIES AND PLANT GROWTH

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In the production of wood pulp for paper or rayon manufacture, wood chips are digested with sulfite leach liquor. The leachate, which contains large amounts of lignin, sulfur, and some sugars, as well as various other substances, is termed "waste sulfite liquor." Disposal of this waste is a serious problem. Various investigations directed toward possible utilization of this material have met with only moderate success.

Phillips et al. (6) point out that the annual discharge from wood pulp mills in this country contains about 1,500,000 tons of lignin, which is equivalent to some 27,000,000 tons of liquor. This material carries a large quantity of combined sulfur, calcium, and potassium, and other substances in varying quantities. The approximate specific gravity of the liquor is 1.12, and the loss from drying is about 89 per cent (5). As may be seen from table 1, waste sulfite liquor contains considerable material that should affect soil fertility and the growth of plants.

Though these data are subject to large variation and error, they serve to give an idea of the quantity of waste liquor and its contents yearly dumped into streams, in many instances causing damage as well as loss of materials that might be recovered and utilized.

The bulkiness of the waste product probably prohibits the use of the material in the raw state as a fertilizer. There is considerable interest, however, in knowing what the effect is when the liquor, in the raw state, is added to the soil. The relatively high sulfur content of the liquor lends interest to the question of using the material on sulfur-deficient soils. Likewise, the high acidity suggests investigation of the possibility of using the liquor for correcting alkali soils. Data on this latter problem will be presented at a later date.

Use of the raw liquor on soils is commonly regarded as undesirable because of toxic effects on plants and microorganisms, although no experimental work to support this claim has been reported. Investigations to determine the specific effects of raw waste sulfite liquor on plant growth, solubility of soil

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nutrients, and microbial processes in the soil were carried out on Newberg loam, a slightly acid soil of high natural fertility.

METHODS

Soils were treated with varying amounts of liquor in cans in the greenhouse to determine the toxic concentration. The cans held 1 pound of soil. Sunflowers were grown as indicator plants and were allowed to germinate and come through the soil before the liquor was applied. In treating with liquor, 2 cc. was put on the soil after seed germination, and the treatment was doubled or tripled weekly until the desired amount of liquor had been added. After a 6-week period of growth, the plants were measured for height and then were harvested for dry weights. Treatments were in quadruplicate, and five plants were grown in each can, giving 20 plants for each treatment.

TABLE 1
*Approximate composition of waste sulfite liquor**

	PER CENT	TONS
Total solids	10.7	2,889,000
Sulfur	0.98	264,600
Calcium	0.48	129,600
Potassium	0.031	5,370
Nitrogen	0.004	1,155
Lignin	5.53	1,500,000
Phosphorus	0.0017	459
Sugars	1.81	488,700
Carbon	0.93	251,100

* Potassium, phosphorus, calcium, and carbon were determined by J. B. Spulnik on a sample of liquor from the Rayonier pulp mill. Nitrogen was obtained from data by Phillips et al. (6). Other data are from Partansky and Benson (5).

The effect of the liquor on soluble nutrients was determined by treating the soil in the same way and incubating for varying periods without planting. Water extracts (1:5) were used for analysis. Potassium was determined by the cobaltinitrite method (4). The calcium was precipitated as the oxalate and titrated with standard permanganate in the usual way. The sulfate sulfur was precipitated and weighed as barium sulfate. Nitrates were determined by the phenoldisulfonic acid method (1).

The effect of the liquor on biological processes was studied by treating 1-kgm. portions of soil in a respiration apparatus and aerating with CO₂-free air. The carbon dioxide produced by the organisms bringing about the decomposition was determined by the method of Bollen and Ahi (2); the carbon dioxide was absorbed in sodium hydroxide and double titrated with standard sulfuric acid, phenolphthalein and brom phenol blue being used as indicators.

In the microbial counts, peptone-glucose-acid agar was used for molds, and

sodium albuminate agar as described by Fred and Waksman (3) was used for bacteria and actinomycetes. Duplicate plates from appropriate dilutions were poured and the numbers of colonies counted after incubation at 28°C. until the colonies could be distinguished.

The titration curves were made from data obtained by using 10-gm. samples of soil and adding acid, alkali, or liquor as indicated with enough water to make a 1:5 suspension. The pH was measured with a glass electrode after 30 minutes of shaking. Ten grams soil and ten cubic centimeters liquor were mixed together and titrated with alkali in a similar manner.

RESULTS OF STUDY

Effect of w.s.l.² on plant growth

The data in table 2 show that sunflowers grew satisfactorily in Newberg loam with rather high concentrations of w.s.l. There was no toxic effect up

TABLE 2
Effect of w. s. l. on the growth of sunflowers

W. S. L. TREATMENTS	AVERAGE HEIGHT OF PLANTS	DRY WEIGHT OF PLANTS	AVERAGE WEIGHT OF PLANTS	INDEX OF GROWTH
cc.	cm.	gm.	gm.	
Control	38.0	3.54	0.19	41.1
2	40.7	4.12	0.21	44.5
4	45.6	4.97	0.26	51.0
8	45.3	5.32	0.28	51.7
16	43.1	3.58	0.24	47.9
22	41.2	3.18	0.23	45.8
30	32.8	1.14	0.23	38.8
48	28.8	0.76	0.25	35.5

to an addition of 30 cc., which corresponds to 84 tons of liquor per acre. The growth response is shown in the column "index of growth." This figure is calculated by taking the greatest height attained by any treatment (60 cm.) as 100. Other treatments then produce heights which are a certain per cent of the maximum. The greatest dry weight for a single plant (1 gm.) is used in the same way for weight comparisons. The height and weight percentages obtained in this manner are added and divided by two. This figure becomes the index of growth. Table 2 shows that the normal growth of the control under the conditions provided for sunflowers is 41.1. A lower figure than this indicates toxicity. In the 30- and 48-cc. applications, the average weights of the plants are high because only five of the twenty original plants survived, yet the height of these plants shows the toxic effect of the liquor. Can 7

² Throughout this section of the paper, w.s.l. is used as an abbreviation for waste sulfite liquor.

(pl. 1, fig. 1) received a 30-cc. application of w.s.l.; only one stunted plant grew, showing definite toxicity.

The effect of lime and w.s.l. without other nutrient additions to the soil is shown in table 3. Enough lime was added to neutralize the w.s.l. The application of 0.8 gm. of lime without liquor, which is equivalent to 2 tons per acre, definitely increased growth. In the neutralization of the w.s.l. with lime, there was no toxic effect up to and including 30-cc. applications of the liquor. The

TABLE 3
Effect of w. s. l. and lime on growth of sunflowers

W. S. L. TREATMENTS	LIME	AVERAGE HEIGHT OF PLANTS	WEIGHT OF PLANTS	AVERAGE WEIGHT OF PLANTS	INDEX OF GROWTH
cc.	gm.	cm.	gm.	gm.	
Control		38.0	3.54	0.19	41.1
0	0.8	41.4	5.27	0.26	47.5
2	0.04	41.5	5.19	0.29	49.0
4	0.08	43.9	5.20	0.27	50.0
8	0.16	41.1	4.46	0.30	49.2
16	0.32	41.5	4.59	0.24	46.5
22	0.64	39.4	4.79	0.28	46.8
30	1.28	37.6	3.40	0.21	41.8

TABLE 4
Effect of w. s. l. used as a source of sulfur for sunflowers over a 6-week period

TREATMENT	SULFUR SUPPLIED BY LIQUOR	AVERAGE HEIGHT OF PLANTS	DRY WEIGHT OF PLANTS	AVERAGE WEIGHT OF PLANTS	INDEX OF GROWTH
	gm.	cm.	gm.	gm.	
Control.....		38.0	3.54	0.19	41.1
Complete nutrient.....	0.439	52.6	17.98	0.94	90.8
2 cc. w. s. l. S-omit.....	0.022	54.2	14.60	0.73	81.7
4 cc. w. s. l. S-omit.....	0.044	57.0	13.75	0.72	83.5
8 cc. w. s. l. S-omit.....	0.088	55.9	13.88	0.82	87.5
16 cc. w. s. l. S-omit.....	0.176	55.5	13.85	0.77	85.0
22 cc. w. s. l. S-omit.....	0.24	56.7	13.81	0.86	90.2
S-omit.....		47.6	11.38	0.57	68.1

plants in this case were definitely more sturdy, as shown by the weights given in table 3. The heights of the plants increased very little. The index of growth indicates that the 4-cc. application is the most favorable to growth. This corresponds to 11.2 tons of liquor per acre.

Table 4 shows the effect of w.s.l. used as a source of sulfur. The complete nutrient used was a solution containing 0.035 gm. potassium, 0.02 gm. nitrogen as nitrate, 0.0217 gm. phosphorus as phosphate, 0.028 gm. calcium, 0.0168 gm. magnesium, and 0.0224 gm. sulfur as sulfate per 100 cc. A total of 1960 cc.

of this solution was applied during the growing period. The nutrient with sulfur omitted was the same as the complete nutrient except for the omission of the sulfur. Only 1610 cc. of this solution was used during the growing period.

The fertilization with the complete nutrient more than doubled the growth of sunflowers, as shown by the index of growth. The nutrient with sulfur omitted caused an increase in growth of 66 per cent over the control. There was a general increase in yield with increased use of w.s.l. along with the other nutrients. Growth was nearly as good when 22 cc. of liquor was used as a source of sulfur as when sulfur was supplied in the usual way in the complete nutrient. This amount of liquor provides about 0.24 gm. of sulfur. Evidently the liquor was a satisfactory source of sulfur, and less sulfur was needed than was supplied by the complete nutrient.

TABLE 5

Effect of w. s. l. used as source of sulfur and neutralized with lime on growth of sunflowers

TREATMENT	LIME	AVERAGE HEIGHT OF PLANTS	WEIGHT OF PLANTS	AVERAGE WEIGHT OF PLANTS	INDEX OF GROWTH
	gm.	cm.	gm.	gm.	
Control.....		38.0	3.54	0.19	41.1
Complete nutrient.....		52.6	17.98	0.94	90.8
S-omit.....		47.6	11.38	0.57	68.1
S-omit.....	0.8	41.1	7.80	0.39	53.8
S-omit 2 cc. w. s. l.....	0.04	54.6	13.65	0.68	79.5
S-omit 4 cc. w. s. l.....	0.08	50.6	12.98	0.65	74.6
S-omit 8 cc. w. s. l.....	0.16	56.0	12.74	0.91	92.1
S-omit 16 cc. w. s. l.....	0.32	50.6	13.63	0.68	76.1
S-omit 22 cc. w. s. l.....	0.64	54.7	12.73	0.75	83.0

Figure 2, plate 1, shows the growth responses when the liquor served as a source of sulfur. Increase in growth follows increased additions of w.s.l.

Table 5 shows the effect of w.s.l. used a source of sulfur and neutralized with lime. Additions of lime tend to decrease growth in comparison to the unlimed treatments. With the addition of 8 cc. of w.s.l. and 0.16 gm. lime, the index of growth is higher than that with the complete nutrient.

One series of treatments was run in which the w.s.l. with no other treatment was put on the soil immediately after planting. Complete data are not available for this experiment, as greenhouse assistants destroyed the plants. The seed germination and growth were not disturbed by additions up to 16 cc. of w.s.l. With the next higher addition, 32 cc., germination was delayed for 2 weeks, and only one seed grew. Here again w.s.l., when added up to this toxic limit, increased growth. The results of this study are shown in figure 3, plate 1.

Effect of w.s.l. on water-soluble nutrients of soil

The soil, treated with w.s.l. as indicated in table 6, showed an increase in potassium, calcium, and sulfate in the water extract. The nitrate content decreased immediately on the addition of the liquor. Upon incubation, there was a complete loss of nitrate. This may be due to the chemical reaction between the sulfites or sulfides present in the liquor and the soil nitrates. Since there was a decrease in nitrates immediately, this explanation seems reasonable. The total absence of nitrates at the end of the 2-week period may be caused in part by microbial activity. The sugars and other easily decomposable organic compounds in the liquor stimulate the microorganisms which utilize nitrates. The acidity caused by the w.s.l. produces an unfavorable medium for nitrification.

Soluble potassium was increased fivefold by the addition of 16 cc. of w.s.l. Since the potassium content of the liquor is 0.0035 gm. per 1 cc., the liquor added accounts for most of the increase. There was a general increase of

TABLE 6
Effect of w. s. l. on water-soluble nutrients of Newberg loam soil
Results in p.p.m.

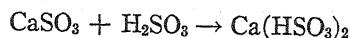
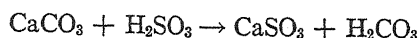
W. S. L. TREATMENT PER 4,000 GM. SOIL	CALCIUM				S AS SO ₄				K				N AS NO ₃			
	0 wk.	2 wk.	5 wk.	10 wk.	0 wk.	2 wk.	5 wk.	10 wk.	0 wk.	2 wk.	5 wk.	10 wk.	0 wk.	2 wk.	5 wk.	10 wk.
cc.																
0	20	27	35	40	2	2	5	3	4	4	6	6	8	8	9	9
2	28	41	44	47	8	20	33	30	5	5	8	8	7	0	0	0
4	100	80	75	61	19	46	61	72	11	13	12	11	7	0	0	0
8	150	145	160	144	26	50	98	148	13	13	16	19	5	0	0	0
16	410	331	262	224	48	120	149	212	20	20	23	25	4	0	0	0

water-soluble potassium on incubation, which may be caused by the slow base-exchange reaction taking place.

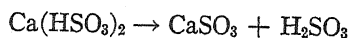
The sulfur present in the w.s.l. is in the form of sulfides, sulfites, sulfates, and organic sulfur compounds. Immediate analysis of soil treated with the liquor is shown in table 6. The increase of the water-soluble sulfates is due to their presence in the liquor. Incubation for 2 weeks doubles the sulfate concentration in almost every treatment. Longer periods of incubation increase the sulfate content, but the increase is not so pronounced as in the initial 2-week period. The sulfur oxidation may be brought about in part by the oxidation of the reduced forms of sulfur by soil air, as well as by the action of sulfur-oxidizing molds and bacteria. Not all of the sulfur added as w.s.l. was converted to sulfates. The maximum conversion after 10 weeks represents about 60 per cent of the total sulfur added in the liquor.

Table 6 shows an immediate increase in the amount of water-soluble calcium.

Since the liquor contains 0.004 gm. calcium per cubic centimeter, the increase was expected. In the heavier treatments, the amount of calcium found exceeds the water-soluble calcium content of the soil and the calcium added in the w.s.l. by a large amount (230 p.p.m. excess for the 16-cc. treatment in original analysis). The acidity of the liquor when added to the soil causes a base replacement, freeing calcium from the colloidal complex by substitution with hydrogen. On the other hand, incubation decreases the soluble calcium in every treatment. This reversal may be explained on the basis of the solubility of calcium sulfite in water. In the formation of sulfite leach liquor, which is used in the extraction of cellulose from wood, limestone is treated with sulfurous acid. The following equations indicate the reaction:



The solubility of $\text{CaSO}_3 \cdot 2\text{H}_2\text{O}$ is but 0.0043 gm. per 100 gm. water at 18° C. (7). The CaSO_3 is highly soluble in sulfurous acid. In w.s.l. there is enough free sulfurous acid to keep the calcium sulfite in solution. This is the case in the immediate analysis of the treated soil. On incubation free H_2SO_3 is oxidized, and the pH of the treated soil increases (table 8). This decrease of acidity would cause the following reaction:



This forms the insoluble calcium sulfite.

Effect of w.s.l. on the biological activity of soil

Carbon dioxide evolution from Newberg loam treated with w.s.l., wheat straw, and calcium nitrate is shown in figure 1. The carbon dioxide evolved per day is given in milligrams of carbon. Since the greatest part of the carbon in the soil is present in the organic matter, the carbon dioxide evolved is used as an indication of organic matter decomposition and biological activity. The results show that any addition of other organic substances with the sulfite liquor increases the amount of carbon evolved in the earlier period of incubation. Upon further incubation, the curves begin to level off, and an equilibrium is reached. Additions of 5 and 10 cc. of w.s.l. increase the carbon dioxide evolved over the control. This is due to the easily decomposed organic substances, such as the sugars, contained in the w.s.l. Calcium nitrate added to Newberg loam decreases the normal evolution of carbon dioxide, a reaction which may be caused by changing the biological flora. A shortage of easily decomposable organic matter may permit ascendancy of autotrophes, which consume carbon dioxide. The addition of w.s.l. and calcium nitrate, however, increases carbon dioxide production over that of w.s.l. alone. In this case, carbohydrates which are readily attacked by the microorganisms

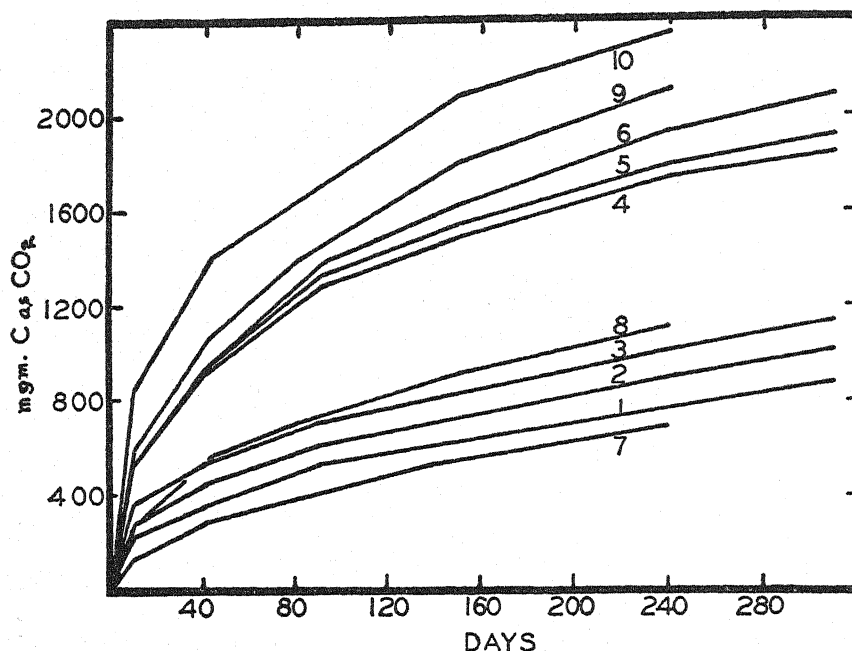


FIG. 1. EFFECTS OF TREATMENTS ON CARBON DIOXIDE EVOLVED FROM NEWBERG LOAM

Rates of Application per kilogram of soil: 1. Control; 2. 5 cc. w.s.l.; 3. 10 cc. w.s.l.; 4. 4 gm. wheat straw; 5. 5 cc. w.s.l. and 4 gm. wheat straw; 6. 10 cc. w.s.l. and 4 gm. wheat straw; 7. 0.88 gm. $\text{Ca}(\text{NO}_3)_2$; 8. 10 cc. w.s.l. and 0.88 gm. $\text{Ca}(\text{NO}_3)_2$; 9. 4 gm. wheat straw and 0.88 gm. $\text{Ca}(\text{NO}_3)_2$; 10. 10 cc. w.s.l., 4 gm. wheat straw, and 0.88 gm. $\text{Ca}(\text{NO}_3)_2$.

TABLE 7

Molds, bacteria, and actinomyces in Newberg loam treated with wheat straw, w. s. l., and calcium nitrate

TREATMENT PER KGM. SOIL	10 DAYS		240 DAYS		310 DAYS	
	Molds*	Actino- myces and bac- teria†	Molds*	Actino- myces and bac- teria†	Molds*	Actino- myces and bac- teria†
Control.....	85	175	210	48		
5 cc. w. s. l.....	115	700			260	37
10 cc. w. s. l.....	70	250			385	97
4 gm. straw.....	78	1,000			450	49
5 cc. w. s. l. + 4 gm. straw.....	150	1,250			420	40
10 cc. w. s. l. + 4 gm. straw.....	170	320			655	83
0.88 gm. $\text{Ca}(\text{NO}_3)_2$ + 4 gm. straw.....	125	240	750	190		
0.88 gm. $\text{Ca}(\text{NO}_3)_2$	75	800	440	61		
10 cc. w. s. l. + 0.88 gm. $\text{Ca}(\text{NO}_3)_2$	120	115	800	55		
10 cc. w. s. l. + 0.88 gm. $\text{Ca}(\text{NO}_3)_2$ + 4 gm. straw.....	95	155	850	155		

* $\times 500$. † $\times 50,000$.

have been added. This is shown by the addition of straw and calcium nitrate, which increases the carbon dioxide evolved over that of wheat straw alone. Wheat straw, calcium nitrate, and w.s.l., added in combination to Newberg loam, produce the most carbon dioxide, especially in the earlier period of incubation.

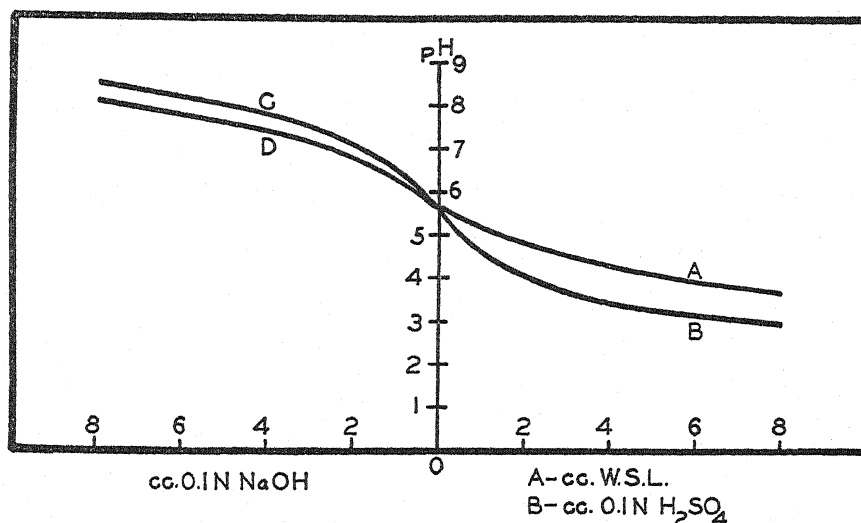


FIG. 2. TITRATION CURVES OF NEWBERG LOAM

A—10 gm. soil titrated with w.s.l.; B—10 gm. soil titrated with 0.1 *N* H₂SO₄; C—10 gm. soil titrated with 0.1 *N* NaOH; D—10 gm. soil and 10 cc. w.s.l. titrated with 0.1 *N* NaOH.

TABLE 8
Effect of w. s. l. on the pH of Newberg loam

W. S. L. TREATMENT PER 400 GM. SOIL	0 WEEKS	2 WEEKS	5 WEEKS	10 WEEKS
cc.				
0	5.7	5.8	5.8	5.8
2	5.3	5.4	5.7	5.8
4	5.1	5.25	5.5	5.6
8	4.9	5.1	5.25	5.4
16	4.7	4.9	5.1	5.3

The effect of treating Newberg loam with wheat straw, calcium nitrate, and w.s.l. on the numbers of molds, bacteria, and actinomyces is given in table 7. The original soil, having a moisture content of 20 per cent and a saturation capacity of 60 per cent, had 7,250,000 bacteria and actinomyces and 38,000 molds. Ten days after treatment, the number of molds had increased in all but the 10-cc. w.s.l., calcium nitrate, and wheat straw treatments. After a longer period of incubation the number of molds had increased in all treat-

ments. In case of the w.s.l. treatments, the number of molds at the end of the incubation period was large because molds are not inhibited by the acidity of the liquor. The increase in bacteria and actinomyces during the first 10 days corresponds to the increase in carbon dioxide produced, except in the calcium nitrate treatment. At the end of the incubation period, the number of bacteria and actinomyces had fallen off to approximately half the original count in each case, showing a tendency of the soil to come to an equilibrium.

Effect of w.s.l. on soil reaction

The w.s.l. used in these experiments has a pH of 1.0. When soil is treated with a solution of such high acidity, the soil reaction is quickly changed. The titration curves are given in figure 2. Newberg loam titrated with 0.1 *N* sulfuric acid maintains a lower pH than when titrated with w.s.l. When soil treated with 10 cc. w.s.l. is titrated with 0.093 *N* sodium hydroxide, 2.5 cc. of sodium hydroxide must be added to reach pH 7, which corresponds to 0.0091 gm. sodium hydroxide or 0.012 gm. calcium carbonate.

Table 8 gives the effect of w.s.l. on the pH of Newberg loam when incubated at room temperature. The pH of the soil drops with increased additions of w.s.l. The return to the normal pH is rapid with the lighter applications. With the 8- and 16-cc. treatments the pH is almost back to normal after 10 weeks. The increase in pH is probably due to the base-exchange reaction of the sulfurous acid with the colloidal matter of the soil, resulting in slightly soluble and slightly ionized acids. This effect is seen in table 6 when, because of base exchange, the amount of water-soluble calcium is increased by w.s.l. additions.

SUMMARY

Waste sulfite liquor in concentration below 80 tons per acre was not toxic to sunflowers grown in Newberg loam. It proved a satisfactory source of sulfur for sunflowers grown on sulfur-deficient soils, definite increases in yield being obtained.

Waste sulfite liquor increased the concentration of potassium, calcium, and sulfate salts in the water extract of the soil and decreased the nitrate content.

Waste sulfite liquor increased the rate of carbon dioxide evolution in the soil, indicating an increased organic decomposition, and increased the microbial population in the soil.

Waste sulfite additions to Newberg loam soil lowered the pH of the soil suspension; on incubation the pH gradually increased.

REFERENCES

- (1) Association of Official Agricultural Chemists. 1919 Official and Tentative Methods of Analysis, ed. 4. Washington, D. C.
- (2) BOLLEN, W. B., AND AHI, S. M. 1938 Effect of alkali salts on general microbial function in the soil. *Soil Sci.* 46: 287-305.

- (3) FRED, E. B., AND WAKSMAN, S. A. 1922 A tentative outline of the plate method for determining the numbers of microorganisms in the soil. *Soil Sci.* 14: 27-28.
- (4) HIBBARD, P. L., AND STOUT, P. R. 1933 Estimation of potassium by titration of the cobaltinitrite with potassium permanganate. *Jour. Assoc. Off. Agr. Chem.* 16: 137-140.
- (5) PARTANSKY, A. M., AND BENSON, H. K. 1936 Methods of analysis of sulphite waste liquor. *Paper Trade Jour.* 102: 29-35.
- (6) PHILLIPS, M., GOSS, M. J., BROWN, B. E., AND REID, F. R. 1936 The ammoniation of waste sulfite liquor and its possible utilization as a fertilizer material. *Jour. Agr. Res.* 53: 209.
- (7) SEIDELL, A. 1919 Solubilities of Inorganic and Organic Compounds, ed. 2. D. Van Nostrand Co. New York.

PLATE 1

FIG. 1. EFFECT OF W.S.L. ON GROWTH OF SUNFLOWERS

1—Control; 2—2 cc. w.s.l.; 3—4 cc. w.s.l.; 4—8 cc. w.s.l.; 5—16 cc. w.s.l.; 6—22 cc. w.s.l.; 7—30 cc. w.s.l.

FIG. 2. EFFECT OF W.S.L. AS A SOURCE OF SULFUR FOR SUNFLOWERS

1—control; 2—nutrient, sulfur omitted; 3—2 cc. w.s.l. and nutrient, sulfur omitted; 4—4 cc. w.s.l. and nutrient, sulfur omitted; 5—8 cc. w.s.l. and nutrient, sulfur omitted; 6—16 cc. w.s.l. and nutrient, sulfur omitted; 7—complete nutrient.

FIG. 3. EFFECT OF W.S.L. ON SEED GERMINATION AND GROWTH OF SUNFLOWERS

1—control; 2—4 cc. w.s.l.; 3—8 cc. w.s.l.; 4—16 cc. w.s.l.; 5—32 cc. w.s.l. All liquor applied at time of seeding.

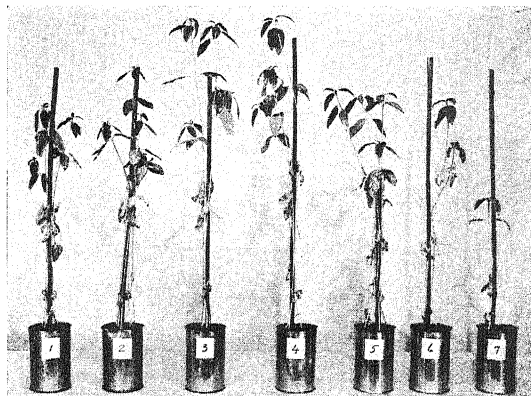


FIG. 1

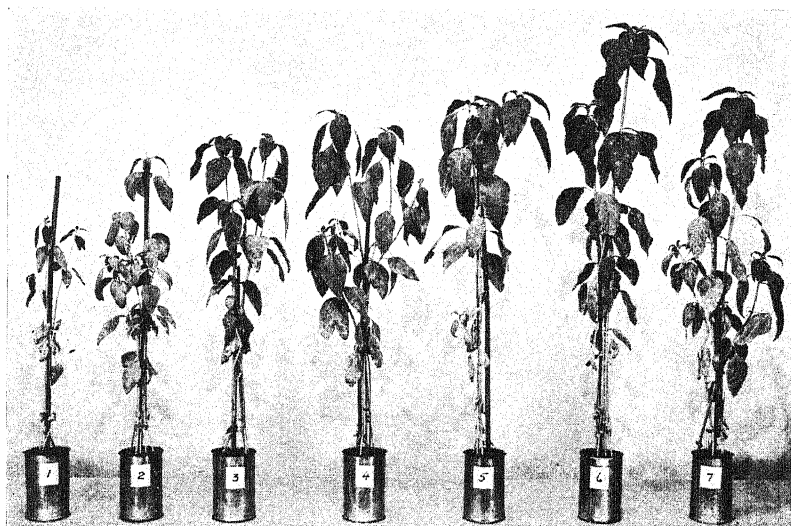


FIG. 2

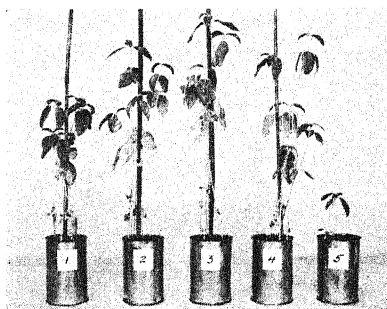


FIG. 3

A SIMPLE APPARATUS AND PROCEDURE FOR THE DETERMINATION OF THE CARBON CONTENT OF THE SOIL¹

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Although the dry combustion method is regarded as standard procedure, many soil scientists are dissatisfied with it (6). Numerous attempts (1, 2, 3, 7, 8, 9, 11, 12, 13, 14) have been made to find a suitable wet oxidation method for the measurement of the carbon content (organic matter) of the soil. Of the oxidizing agents available, dichromate and iodate are outstanding (5). Dichromate has been used extensively for this determination. Most of the workers who have employed this reagent, however, failed to consider the fact that most organic compounds are not completely oxidized under the conditions reported and that considerable carbon monoxide and free oxygen are evolved during the reaction. It is surprising that iodate, which does not possess these limitations, has received little attention.

After a survey of the present methods it is the opinion of the authors that the complicated apparatus (particularly for pretreating evolved gases and measuring carbon dioxide) and failure to attain complete combustion were among the greatest limitations to the present wet combustion methods. For this reason a simple apparatus [employing the Pettenkofer principle (10, p. 221)] and procedure using either dichromate or iodate has been devised for the measurement of either the carbon content or both the reducibility and the carbon content of the soil.

APPARATUS

The apparatus is illustrated diagrammatically in figure 1. The reaction vessel *A* (total volume 20 ml.) was constructed from a No. 15 standard taper joint and a capillary stopcock. The slow combustion chamber *B* consists of a three-way stopcock, a slow combustion unit (as used in gas analysis), and a leveling bulb. Mercury is used as the confining fluid. The reaction flask *C* for the analysis of carbon dioxide was designed from a 250-ml. Erlenmeyer flask, a No. 20 standard taper joint, and a capillary stopcock.

A tubular rheostat (not shown) is connected to a 110-volt circuit. This,

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when used as a potential divider, provides a suitable source of current for heating the platinum spiral of the slow combustion unit.

PROCEDURE

The reaction vessel *A* is charged with 250 mgm. of soil and 250 mgm. of dichromate or iodate, and the system is flushed with CO₂-free air. Closing the capillary stopcock *a* and opening the three-way *b* permits the flow of emitted gases into the slow combustion chamber, which is maintained at a slight vacuum by means of the leveling bulb. The carbon dioxide flask is charged with 10 ml. of standard 0.25 *N* Ba(OH)₂ (measured with an automatic pipette), partially

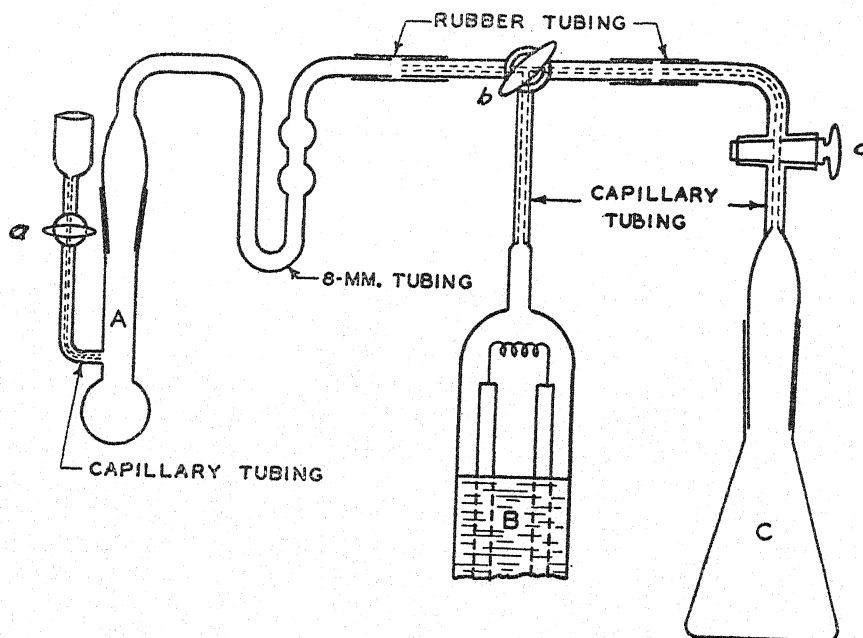


FIG. 1. APPARATUS FOR DETERMINING THE CARBON CONTENT OF THE SOIL

evacuated, and then connected to the other arm of the three-way stopcock. Three milliliters of concentrated (carbon-free) sulfuric acid is introduced into the reaction vessel by means of stopcock *a*. The charge is now ready for combustion.

The stopcock *a* and the standard taper joint of the reaction vessel *A* are lubricated with sirupy phosphoric acid. By means of a phosphoric acid bath (4) the temperature of the reaction chamber is kept between 160 and 80°C. for 20 minutes. The gases which are generated during this time pass through the U-tube containing 1 ml. of 0.5*N* KI solution to the slow combustion chamber *B*. At the completion of the heating operation stopcock *a* is opened and 5 ml. of 6 *N* H₂SO₄ plus sufficient water to fill all but a small

part of the free air space is introduced. An Ascarite tower is then connected to the apparatus through stopcock *a*, and sufficient carbon dioxide air is flushed through to sweep all the carbon dioxide into the slow combustion chamber. The coil is then brought to a fairly red heat for 2 minutes, and the gases are transferred to the reaction flask *C*. Reaction flask *C* is then directly connected through stopcocks *a* and *b* to a source of CO₂-free air and brought to atmospheric pressure. It is then removed and allowed to stand for 20 minutes. Five milliliters of acetone is then introduced to improve the end point, and the excess barium hydroxide is titrated with 0.05 *N* HCl to the thymol blue end point. The amount CO₂ formed can be calculated from these data.

TABLE 1
Results of determination of carbon contents of pure compounds

SUBSTANCE	WEIGHT OF SAMPLE	CARBON CONTENT	
		Measured	Calculated
	<i>mgm.</i>	<i>per cent</i>	<i>per cent</i>
Succinic acid.....	16.34	40.66	40.68
	16.38	40.71
	18.44	40.67
Hydroquinone.....	14.19	65.34	65.44
	11.76	65.46
Benzoic acid.....	10.04	68.84	68.44
	12.94	69.05
Vanillin.....	16.47	63.22	63.15
	12.13	63.18
Sucrose.....	15.69	41.94	42.11
	13.09	42.08

To prevent the diffusion of air into the flask during the titration, a rubber sheet (such as that used for a dental dam) was loosely fitted over the mouth of the flask. The top of the burette was inserted through a small hole in the rubber. This arrangement kept out all the air without complicating the procedure.

When using iodate as the oxidant, charges of the same size are employed. The time of heating is increased to 30 minutes, and the temperature is kept between 185 and 195°C. The use of the coil in this case is unnecessary, and only 5 ml. of 6 *N* H₂SO₄ is introduced at the end of the heating period.

To determine the oxygen consumption, the reaction vessel *A* is removed, and the mixture is transferred to a 1-liter Erlenmeyer flask, then diluted to approximately 100 ml. and steamed until disappearance of the iodine color. Sufficient Na₂CO₃ to neutralize about all but 1 ml. of the concentrated H₂SO₄

TABLE 2
Carbon content of soils

SAMPLE NUMBER	TYPE*	C MEASURED	C CALCULATED FROM KIO ₃ USED	ORGANIC MATTER FROM CO ₂	ORGANIC MATTER O.S.C. EXP. STA.
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	Umatilla				
19064	SCL	2.95	2.98	5.09	5.17
19065	SCL	1.24	1.25	2.14	2.33
19066	SCL	0.55	0.63	0.96	0.99
	Rupert				
19076	Crs.S	1.66	1.78	2.87	3.26
19077	Crs.S	0.80	0.84	1.38	1.65
19078	Crs.S	0.56	0.58	0.95	1.09
	Palouse				
19116	Si.L	2.26	2.33	3.90	4.00
19117	Si.L	2.44	2.50	4.04	4.42
19118	Si.L	2.35	2.62	4.05	4.09
	Umatilla				
19162	Onyx L	1.08	1.37	1.86	2.07
19163	Onyx L	0.56	0.89	0.97	1.21
	Josephine				
19903	GL	1.16	1.22	2.05	1.92
19904	GL	0.54	0.57	0.93	0.74
19905	GL	0.34	0.42	0.59	0.41
	Josephine				
19906	FSL	0.91	0.94	1.57	1.38
19907	FSL	0.63	0.62	1.09	0.81
19908	FSL	0.28	0.29	0.49	0.41
	Salem				
19909	SL	1.21	1.19	2.08	1.95
19910	SL	0.60	0.58	1.04	0.91
19911	SL	0.34	0.34	0.58	0.62
	Josephine				
19912	GL	2.16	2.25	3.73	3.52
19913	GL	0.25	0.31	0.44	0.45
	Umatilla				
19061	L	1.97	2.21	3.40	3.34
	Pilot Rock				
19067	Si.L	0.99	1.32	1.70	1.82
	Walla Walla				
19070	Si.L	1.00	1.34	1.73	1.85

* S = sandy or sand; C = clay; L = loam; Crs. = coarse; F = fine; Si. = silty; G = gravelly.

is added. The solution is then titrated with 0.1 N $\text{Na}_2\text{S}_2\text{O}_3$. This measured the excess iodate.

The amount of iodate used was found to be consistently greater than the theoretical, because of traces of organic matter in the H_2SO_4 and thermal decomposition. This necessitated the use of a blank, which amounted to 0.90 mgm.

For reasons cited, the authors recommend the use of iodate in preference to dichromate for this measurement.

RESULTS AND DISCUSSION

In order to check the precision of this method the carbon contents of a number of pure organic compounds were determined. The results of these experiments are shown in table 1.

It is apparent from the results of table 1 that this procedure is capable of a high degree of precision.

After the procedure was checked against pure compounds, the carbon content and oxygen consumed values of a number of soil samples were determined. The results of iodate experiments are shown in table 2.

The percentage of carbon calculated from KIO_3 consumed was based on the assumption that the soil organic matter was carbohydrate in nature (15). The principal sources of error which interfere with the measurement of reducible organic matter are the presence of unoxidized inorganic constituents and halides in the soil.

Although the determination of evolved CO_2 is much more accurate than oxygen consumed data, the use of both sets of data shows promise as an index of the state of oxidation of soil organic matter. Abnormal values (either high or low) at least indicate the presence of large amounts of reduced inorganic components or carbonates in the soil.

The apparatus described can be used for a number of measurements using either dichromate or iodate as the oxidizing agency.

As a means of measuring the carbon content of the soil this procedure requires the minimum of time and operative skill. Furthermore, it eliminates the errors due to formation of carbon monoxide or presence of halides.

REFERENCES

- (1) ALEXANDER, L. T., AND BYERS, H. G. 1932 A critical laboratory review of methods of determining organic matter and carbonates in soil. *U. S. Dept. Agr. Tech. Bul.* 317: 1-25.
- (2) ALLISON, L. E. 1935 Organic soil carbon by reduction of chromic acid. *Soil Sci.* 40: 311-320.
- (3) ANDERSON, M. S., AND BYERS, H. G. 1934 The carbon-nitrogen ratio in relation to soil classification. *Soil Sci.* 38: 121-138.
- (4) CHRISTENSEN, B. E., AND KING, A. E. 1936 An inorganic liquid mixture for temperature baths in range 100-250°. *Indus. and Engin. Chem., Analyt. Ed.* 9: 194.
- (5) CHRISTENSEN, B. E., WILLIAMS, R. J., AND KING, A. E. 1937 Organic oxidation equivalent analysis: III. *Jour. Amer. Chem. Soc.* 59: 293.

- (6) CROWTHER, E. M. 1935 First report of the organic carbon committee. *Trans. Third Internat. Cong. Soil Sci.* 1: 114-127.
- (7) DEGTJAREFF, W. TH. 1930 Determining soil organic matter by means of hydrogen peroxide and chromic acid. *Soil Sci.* 29: 239-245.
- (8) HECK, A. F. 1929 A method for the determination of total carbon and also for the estimation of carbon dioxide evolved from soils. *Soil Sci.* 28: 225-233.
- (9) KING, N. J. 1932 Wet and dry combustion methods for determining the total carbon in soils and other materials. *Chem. Engin. Mining Rev.* 24: 429.
- (10) LUNGE, G., AND AMBLER, H. R. 1934 Technical Gas Analysis. Gwiney and Jackson, London.
- (11) MARTIN, W. S., AND GRIFFITH, C. 1935 The determination of carbon in soils by wet combustion method. *Jour. Soc. Chem. Indus.* 54: 234-235 T.
- (12) NICLOUX, M. 1930 The determination of carbon in vegetable soils. Carbonate carbon and organic carbon. *Ann. Sci. Agron.* 47: 384.
- (13) ROBINSON, G. W., McLEAN, W., AND WILLIAMS, R. J. 1929 The determination of organic carbon in soils. *Jour. Agr. Sci.* 19: 315.
- (14) SCHOLLENBERGER, C. J. 1927 A rapid approximate method for determining soil organic matter. *Soil Sci.* 24: 65-68.
- (15) TYURIN, I. V. 1935 A method for the simultaneous determination of organic carbon and the "oxidation value" of soil organic matter. *Trans. Third Internat. Cong. Soil Sci.* 1: 111-113.

A RAPID METHOD OF CHECKING THE ACCURACY OF REPORTED WATER ANALYSES¹

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It is well known that the specific electrical conductance of natural waters is a rough measure of the total concentration of electrolytes present in solution. These electrolytes are, in the majority of cases, varying mixtures of chlorides, sulfates, bicarbonates, carbonates, and in some instances nitrates, and of sodium, magnesium, and calcium. Other electrolytes are present in amounts too small, as a rule, to be important quantitatively.

TABLE 1

Mean ratios of total cations to specific electrical conductance in waters of varying sodium and chloride percentages

PER CENT SODIUM IN TOTAL CATIONS	$k \times 10^5$ AT 25°C., 25 TO 150					$k \times 10^5$ AT 25°C., 150 TO 250					$k \times 10^5$ AT 25°C., 250 TO 350*				
	Per cent Chloride in Total Anions														
	Below 20	20-40	40-60	60-80	Over 80	Below 20	20-40	40-60	60-80	Over 80	Below 20	20-40	40-60	60-80	Over 80
	Ratios of Total Cations to Conductance														
	Below 20	0.115	0.111	0.106	0.102	0.098	0.119	0.114	0.109	0.104	0.100	0.122	0.116	0.111	0.106
20-40	0.110	0.106	0.102	0.098	0.094	0.114	0.109	0.104	0.100	0.096	0.116	0.111	0.106	0.102	0.098
40-60	0.105	0.102	0.098	0.094	0.091	0.109	0.104	0.100	0.096	0.093	0.111	0.106	0.102	0.098	0.094
60-80	0.101	0.098	0.094	0.091	0.088	0.104	0.100	0.096	0.093	0.089	0.106	0.102	0.098	0.094	0.091
Over 80	0.097	0.094	0.091	0.088	0.085	0.100	0.096	0.093	0.089	0.086	0.102	0.098	0.094	0.091	0.088

* For every successive increase of 100 in $k \times 10^5$ at 25°C. over 350, add 0.0025 to the factor.

In a study of several thousand irrigation and drainage water analyses, it has been found that the ratio of the sum of the cations or anions (milliequivalents per liter) to the specific electrical conductance at 25°C. (reciprocal ohms $\times 10^5$) falls ordinarily between the values of 0.085 and 0.122. Further observations have indicated that the variations between these limits are affected primarily by (a) the ratio of sodium to total cations, (b) the ratio of chloride to total anions, and (c) the total concentration of electrolytes.

These observations suggest a means of checking the accuracy of complete analyses, and of calculating the sum of calcium plus magnesium on the basis of three determinations; namely, (a) conductance, (b) sodium, and (c) chloride.

¹ Paper No. 403, University of California Citrus Experiment Station, Riverside, California.

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The method here outlined is in no sense offered as a substitute for complete analysis, but primarily as a means of detecting errors. In cases where it is desired to secure a rough estimate of the probable amounts of calcium plus magnesium, the method is applicable, provided it is known that cations other than calcium, magnesium, or sodium are not present in appreciable quantities.

A series of factors relating conductance to total electrolytes in waters of varying chloride and sodium percentages are shown in table 1. These represent averages derived from a large number of accurate water analyses. In table 2 are shown calculated versus determined values of the total cations and of calcium plus magnesium for a number of water samples of widely varying composition.

The method of computing the total concentration of electrolytes and of calcium plus magnesium on the basis of determinations of conductance, sodium, and chloride can be illustrated by the following examples:

1. Water sample S-179, San Gabriel River (table 2). Determined values:

$$k \times 10^5 \text{ at } 25^\circ\text{C.} = 65.4 \text{ ohms}^{-1}$$

$$\text{Sodium} = 1.00 \text{ m.e. per liter}$$

$$\text{Chloride} = 0.38 \text{ m.e. per liter}$$

The total cation or anion content of this water will lie between values of 5.56 and 7.98 m.e. per liter: that is,

$$65.4 \times 0.085 = 5.56$$

$$65.4 \times 0.122 = 7.98$$

Hence, sodium constitutes between 12 and 17 per cent of total cations; and chloride, between 5 and 7 per cent of total anions. Since both sodium and chloride constitute less than 20 per cent of total cations and anions, respectively, the appropriate factor is 0.115 (table 1).

Total cations:

$$65.4 \times 0.115 = 7.52 \text{ m.e. per liter}$$

Calcium plus magnesium:

$$7.52 - 1.00 = 6.52 \text{ m.e. per liter}$$

2. Water sample J-17, Ground water, Silaxo, San Joaquin Valley (table 2). Determined values:

$$k \times 10^5 \text{ at } 25^\circ\text{C.} = 926 \text{ ohms}^{-1}$$

$$\text{Sodium} = 52.7 \text{ m.e. per liter}$$

$$\text{Chloride} = 62.5 \text{ m.e. per liter}$$

The total cation or anion content of this water will lie between values of 126 and 92 m.e. per liter: that is,

$$926 \times \left(0.122 + \left[\frac{926 - 350}{100} \times 0.0025 \right] \right) = 126$$

$$926 \times \left(0.085 + \left[\frac{926 - 350}{100} \times 0.0025 \right] \right) = 92$$

TABLE 2

Comparison between calculated and determined concentrations of total cations and of calcium plus magnesium

SAMPLE NUMBER	SOURCE	DETERMINED VALUES			TOTAL CATIONS		CALCIUM PLUS MAGNESIUM	
		$k \times 10^5$ at 25°C.	Sodium	Chloride	Determined	Calculated	Determined	Calculated
		ohms ⁻¹	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.	m.e./l.
8028	Sacramento River	19.4	0.52	0.29	2.13	2.12	1.60	1.61
11833	Gridley Well, Coachella	23.0	2.01	0.30	2.39	2.23	0.38	0.22
H-80	Rowell Well, Thermal	26.3	1.44	0.35	2.74	2.76	1.30	1.32
S-144	Union Well, Fontana	30.5	0.41	0.20	3.22	3.51	2.81	3.10
H-60	Agua Caliente Hot Spring	35.6	3.04	1.00	3.22	3.35	0.18	0.31
S-188842*	Warm Spring, San Bernardino	36.0	2.22	0.45	3.68	3.71	1.46	1.49
S-3	Whittier Narrows	43.5	0.92	0.30	4.55	4.59	3.63	3.67
S-5908*	Los Angeles Aqueduct	45.0	2.35	0.70	4.59	4.72	2.24	2.37
S-148	Osbum Well, San Bernardino	55.6	3.31	0.66	5.60	5.72	2.29	2.41
S-4801A*	Sulphur Spring, San Fernando	68.6	2.30	0.90	7.42	7.55	5.12	5.25
S-179	San Gabriel River	65.4	1.00	0.38	7.48	7.52	6.48	6.52
E-103-c*	Medical Evangelists Well, Loma Linda	69.6	3.61	1.22	7.49	7.34	3.88	3.73
S-61	Sasoku Well, Newport Beach	76.4	7.06	2.82	7.52	7.19	0.46	0.13
D-762*	Bird Well, Chino	94.1	1.04	4.51	9.78	9.97	8.74	8.93
S-79	El Segundo City Well	105.	3.36	5.34	11.9	10.7	8.54	7.34
S-105	Victoria Well, Puente	108.	3.18	1.64	11.7	11.9	8.52	8.72
S-193	San Jose Creek	132.	3.75	1.51	14.8	14.5	11.1	10.8
S-238	Los Angeles River	135.	5.65	1.50	15.1	14.9	9.42	9.25
J-3	Ground Water, Los Banos	147.	11.7	3.30	14.7	14.4	2.97	2.71
J-8	Ground Water, Los Banos	154.	14.2	1.39	14.5	14.9	0.28	0.70
J-22	Ground Water, Romero, San Joaquin Valley	159.	6.25	5.95	16.9	16.3	10.7	10.1
H-85	Wise Flowing Well, Mecca	171.	10.6	0.63	17.3	17.4	6.70	6.80
B-16*	Investment Co. Well, Los Angeles	190.	3.57	10.0	20.1	20.1	16.5	16.5
11824	Hittson Cold Spring, Coachella Valley	205.	16.5	4.31	20.6	20.5	4.08	4.00
C-853-w	School Well, near Santa Fe Springs	209.	4.26	13.0	21.4	21.3	12.1	12.0
12069	Dos Palms Well near Salton Sea	340.	24.6	24.3	31.7	32.0	7.10	7.40
S-12156A*	Spring, South of Riverside	403.	21.0	25.8	41.3	40.7	20.3	19.7
B-6-K*	Beverly Hills Well	472.	14.1	38.4	49.5	49.5	35.4	35.4
S-187	Edwards Drain, Huntington Beach	504.	26.9	40.6	47.1	47.4	20.2	20.5
J-17	Ground Water, Silaxo, San Joaquin Valley	926.	52.7	62.5	107.	108.	54.3	55.3
J-35	Ground Water, Silaxo, San Joaquin Valley	1180.	101.	30.2	155.	150.	54.0	49.0
S-11	Irvine Drain, Santa Ana	2120.	185.	58.0	277.	307.	92.0	122.

* Analyses taken from Bulletin 40A, California Division of Water Resources, Sacramento, 1936.

(See table 1 footnote.) Hence, sodium constitutes between 42 and 57 per cent of total cations; and chloride, between 50 and 68 per cent of total anions. The factor to be employed is, accordingly, either 0.1164 or 0.1124 (table 1): that is,

$$0.102 + \left(\frac{926 - 350}{100} \times 0.0025 \right) = 0.1164$$

$$0.098 + \left(\frac{926 - 350}{100} \times 0.0025 \right) = 0.1124$$

By a second and closer approximation, the total cation or anion content of this water is between 108 and 104 m.e. per liter: that is,

$$926 \times 0.1164 = 108$$

$$926 \times 0.1124 = 104$$

By this approximation, therefore, sodium constitutes between 49 and 51 per cent of total cations; and chloride, between 58 and 60 per cent of total anions. Hence, the appropriate factor is 0.1164 rather than 0.1124.

Total cations:

$$926 \times 0.1164 = 108 \text{ m.e. per liter}$$

Calcium plus magnesium:

$$108 - 52.7 = 55.3 \text{ m.e. per liter}$$

Major compensating errors in the determinations of the cations and anions are especially difficult to discover, for the sum of the determined cations and the sum of the determined anions do not differ greatly one from the other, and the analysis may seem correct when it is not. The ratio of cations to conductance, in this case, is one way of detecting an error. If this ratio does not correspond to the determined values of a given water sample and if the value of conductance is confirmed by duplicate determinations, the entire analysis may be questioned. Too high a ratio indicates major compensating errors, whereas too low a ratio means either major compensating errors or the presence of undetermined constituents, or both. In the experience of the writer, compensating errors of the type described are not infrequent.

SUMMARY

In the analyses of several thousand water samples the author has worked out a series of factors relating conductance to total concentration of dissolved electrolytes. The values of these factors fluctuate chiefly with changes in total electrolyte content and with the percentages of sodium and of chloride as of total cations and anions respectively. Having determined the conductance of a water and its sodium and chloride content, one can, by means of the factors presented, calculate with considerable accuracy the total elec-

trolyte content of a water and the sum of calcium plus magnesium expressed as milliequivalents. The method here described is in no sense offered as a substitute for complete analysis but rather as a means of detecting errors made either in the course of such analyses or in calculating and recording the results. In those cases where a rough estimate of calcium plus magnesium is all that is needed, the method is satisfactory, provided important amounts of bases other than calcium, magnesium, and sodium are absent.

A SOIL ZINC SURVEY IN CALIFORNIA¹

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During recent years it has become generally recognized that an adequate supply of the so-called minor elements in soils, one of which is zinc, is necessary for successful growth of many crops. For several years the writer took part in an investigation of the zinc content of plants particularly in relation to certain plant diseases, such as peach little leaf, pecan rosette, and citrus mottle leaf. This work indicated the desirability of a study of the zinc-supplying power of soils, a study still in progress. During the first two years, a satisfactory procedure for estimating zinc and some other associated metals was developed (4), and numerous samples of soils and associated rocks were collected from many localities. This paper constitutes a report of the results of examination of these samples.

COLLECTION OF SAMPLES

A majority of the samples were taken from places within a few miles of San Francisco Bay: from Berkeley Hills, from the gently sloping ground near the Bay in West Berkeley, from Marin County near Mt. Tamalpais, and from San Bruno Hills south of San Francisco. One group of samples was taken in the delta region of San Joaquin County and in adjoining Contra Costa County. Another group was collected along the highway from Berkeley to Placerville, in Contra Costa, Sacramento, El Dorado, and San Joaquin Counties.

In the collection, preparation, and analysis of the samples, contamination with any source of zinc such as brass or zinc-coated sieves or other apparatus was carefully avoided. In general, it was planned to take samples only from places not likely to have been contaminated by human activities, usually from ground apparently never cultivated, but this plan was not always followed. The purpose has been to get samples from as many different localities as could be visited. Consequently, this may be considered as a reconnaissance survey of some localities which gives some knowledge of the occurrence and amount of zinc in different places and which may serve as a guide for further work.

Though most of the samples are not composites of more than one hole, they are believed fairly to represent the places from which they were taken, except in the case of pieces of rock picked up at random, perhaps far from their original sources.

¹ Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration Official Project #465-03-3-587.

PREPARATION AND ANALYSIS OF SAMPLES

The samples were brought to the laboratory in paper sacks, air dried, pulverized in a steel mortar, and passed through a 40-mesh stainless steel sieve. In addition, most of the rock samples were passed through a silk bolting cloth of about 100 mesh.

Methods of analysis

Zinc, with small amounts of some other minor metals, was extracted from the soils or rocks by action of the standard solvent and equilibrium procedure already described (4). Five grams of the powdered material is placed in a 500-cc. cylindrical glass-stoppered pyrex bottle with 400 cc. of the solvent, which is 0.05 *N* KCl plus enough acetic acid, about 4 cc. glacial per liter, to make the pH 3.2. The stoppered bottle is laid on its side on the "roller" (1) and slowly rotated 4 or 5 hours, then stood on the table overnight to permit sedimentation. Next morning all but 3 to 5 cc. of the clear solution is siphoned off, another 400 cc. of solvent is added, and the mixture is rolled, settled, and siphoned off as before. The two extracts may be analyzed separately, to give some notion of the ease of extraction, or combined then analyzed as a whole to show the total extracted. The solvent and the procedure for extraction were chosen after many months had been spent in making hundreds of experiments to discover the most effective and simple means of extracting the zinc. The solvent used was chosen because it extracts zinc fairly well, dissolves very little iron, and attacks silicates very little. The acidity is made pH 3.2 because that is nearly the highest acidity likely to be found in soils. It was desired to use a solvent which would have solvent power comparable to the soil solution of an acid fertile soil. The KCl has little solvent effect and is useful chiefly as a flocculating agent to cause rapid sedimentation of insoluble matter from the equilibrium extracts. Work previously reported (4) has shown that only acid solvents dissolve much zinc from ordinary soils, and that the greater the concentration of hydrogen ion, the more zinc is extracted. Water or weakly alkaline solvents remove very little zinc from most soils.

Chemical analysis of the soil extracts

The dithizone method was used for extracting zinc and some other metals (4) from the aqueous saline soil extract. Suitable modifications of the dithizone method permit a fairly satisfactory separation of the extracted metals as described elsewhere (3). Since that paper was written some slight changes have been made in the procedure, which briefly described, is as follows:

First, copper is removed from the acid soil extract by repeated extraction with dithizone in chloroform. After removal of the copper, citrate is added to prevent precipitation of iron, manganese, nickel, and cobalt, then ammonia is added to make the pH 8 to 9, and all the other metals removable by dithizone are extracted together. The chloroform solution of the dithizonates is shaken

with 0.05 *N* HCl, which removes zinc, lead, and cadmium if present. So far as is known, the amount of cadmium in ordinary soils is too small for detection by this procedure. The zinc and lead in the acid extract are estimated to-

TABLE 1
Lowest and highest quantities of available zinc found in groups of soils from widely separated localities

GROUP	SAMPLE NUMBERS	DEPTH	ZINC	
			Lowest	Highest
		<i>inches</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
1. Silty clay loams from hills about Berkeley, showing variation within an area 1 mile in diameter and differences between topsoil and subsoil	1-16	1-6	1.9	4.4
		6-20	1.1	2.3
2. Similar to, and from same range of hills 3 to 4 miles south of group 1	49-54	1-6	2.2	5.8
	52	1-6	22.2
	117	0-2	94.0
3. Silty clay loams from somewhat level northwest part of the City of Berkeley near the Bay of San Francisco	40-48	1-20	2.3	7.0
	(7 samples) 38, 39, 43	1-6	12.0	13.3
4. From Moraga Ridge 3 miles east of group 2	119-120	1-6	2.2	5.1
	(shaly loams) 118-122 (high organic forest mull)	0-2	20.0	32.7
5. Leaves and silt loams from Mt. Tamalpais region of Marin County just north of the Golden Gate	55-59 (south side of mountain)	0-6	1.1	7.2
	106-109 (north side of mountain)	0-6	1.6	5.2
6. From San Bruno Hills, just south of San Francisco	32-35 (loams)	0-6	1.2	2.7
	133 (dune sand)	0-6	4.0
7. From east side of lower San Joaquin Valley, nearly level	78-79 (loams)	0-6	0.3	2.0
	91-94 (clay loams near river)	0-6	1.6	5.6
8. Loams and clays from Sierra Nevada foothills, El Dorado County	80-90	0-6	0.3	2.6
9. Cashion Creek area of Contra Costa County, 10 miles east of Berkeley	17-20 (loams)	0-6	1.3	7.1
	98-105 (clay loams)	0-10	2.5	5.6

gether as zinc by the bromine titration method (3). Lead is estimated in a separate portion of the soil extract by addition of potassium cyanide, which suppresses action of dithizone on all the usual soil metals except lead. The

TABLE 2
Samples high in organic matter

SAM- PLE	DEPTH	SAMPLING PLACE	Zn	Pb	Cu	Co	Ni	X
	<i>inches</i>		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
117	0-2	Under old redwood tree	94.0	4.0	5.1	1.0	+
52	6	Same locality as 117	22.2	1.5	+	0.4	3.2
118	0-1½	Under old pine	27.3	20.0	3.2	+	+	9.1
119	6	Same hole as 118	5.1	1.0	+	+	+	6.6
121	0-1	Under redwood tree	32.7	1.5	+	+	+	12.3
122	10	Same hole as 121	10.6	2.0	7.1
126	0-1	Under heavy old grass	8.7	1.5	2.4	5.3
127	8	Same hole as 126	4.0	1.0	+	4.2
129	0-1	Under heavy old grass	12.9	1.0	9.3
130	8-10	Same hole as 129	7.9	1.0	3.9
105	1-2	Under old oak tree	22.0	0.5	10.5
103	10	Same hole as 105	5.1	0.5	4.2
131	0-1	Scanty grass, many small rocks	5.4	0.5	3.1
132	8-10	Same locality as 131	3.1	0.5	2.8

TABLE 3
*Soil and rock from same location**

SAMPLE	TYPE	ZINC
		<i>p.p.m.</i>
{ 34	Silt loam	2.7
{ 34R	Sandstone	2.4
{ 50	Loam	5.8
{ 54	Serpentine rock	13.4
{ 60	Loam	2.1
{ 61	Decayed rock	1.0
{ 79	Loam	2.0
{ 80	Gravel	1.4
{ 86	Holland loam	1.1
{ 87	Granite	2.0

* Four similar pairs not shown.

amount of lead found, usually small, is subtracted from the sum of the lead and zinc, thus giving the amount of zinc. In many soils the lead is so little that it may be neglected without making much difference in the figure for zinc.

In case it is not important to estimate the amounts of the other metals, all may be extracted together from the aqueous solution at pH 8 to 9 by dithizone in chloroform. From this solution zinc is removed, as described, by 0.05 *N* HCl and again separated by dithizone after the solution has been made alkaline.

After removal of the zinc and lead the chloroform contains the dithizonates of cobalt and nickel. The solution is divided into two parts, which are evaporated, the residue is ignited then dissolved in hydrochloric acid, and the metals are estimated by color tests. No satisfactory method of separating nickel and

TABLE 4
Rock, not related to soil samples

SAMPLE	DESCRIPTION	Zn	Pb	Cu	Co	Ni	X
		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
69	Paving rock, U. C. Campus	2.6	1.5
74	Parent rock, Paradise Valley	2.7	3.2	7.0
81	Andesite, near Whitehall	5.0	1.0	46.0	1.0	2.4
97	Andesite, 12 miles east of Lodi	3.2	0.5	5.4
114	Yellow shale	3.2	2.0	1.2	8.4
	Brown shale } two layers in same piece	5.9	2.5	0.8	10.8
115	Brown shale, near 114	16.8	3.0	10.1	0.6	4.8

TABLE 5
Samples containing considerable amounts of other metals besides zinc

SAMPLE	DESCRIPTION	Zn	Pb	Cu	Co	Ni
		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>
38*	Brown loam, West Berkeley	12.4	46.0	9.6	2.4	9.6
43*	Brown loam, West Berkeley	13.3	5.0	2.2	4.0	++
46	Brown loam, West Berkeley	4.6	1.0	3.4	1.4	25.0
54	Serpentine rock	13.4	1.0	3.4	4.0	+++
55	Red loam, Mt. Tamalpais	5.4	2.0	2.8	++	1.6
56	Red loam, Mt. Tamalpais	1.1	9.5	+	+	+
115	Brown shale, Berkeley Hills	16.8	3.0	10.1	0.6	48.0
124	Serpentine rock, Mt. Tamalpais	2.3	1.0	1.1	5.0	32.0
125	Brown loam at side of 124	3.2	2.0	0.6	3.2	64.0

* Perhaps contaminated from nearby habitations.

cobalt by means of dithizone has been found. The color tests are simple and not seriously interfered with by larger amounts of the other metals present.

Nickel is estimated by the Rollet test (6), which produces a brownish yellow color by the action of dimethyl glyoxime on the solution of nickel, which has been oxidized to nickelic by action of bromine.

The cobalt is estimated by means of the Van Klooster nitroso R salt test (7) as modified by Kidsom, et al. (5), but using, instead of a Lovibond tintometer, direct ocular comparison with known amounts of cobalt similarly treated, to measure the amount in the unknown.

The total amount of copper, cobalt, and nickel, after removal of zinc, may be estimated by titration of the chloroform solution of the dithizonates with bromine by the same method as that used for zinc. The amount of cobalt in this titrated solution is indicated by the depth of brown color produced by the bromine, since bromine titrated solutions of all the other metals are nearly colorless. Because the amount of bromine required to oxidize the dithizonates of zinc, copper, nickel, and cobalt is about the same for the same amount of each of these metals, this titration gives an estimate of the total as equivalent

TABLE 6
Regional groups of agricultural soils

SAMPLE	DESCRIPTION	DEPTH	ZINC
		inches	p.p.m.
Coastal hills			
30	Manzanita red loam, near Cowell	24	3.2
31	Zamorra clay, north east of Walnut Creek	12	2.3
34	Brown loam on Colma hills	6	2.7
65	Brown loam at Kentfield	8	2.6
73	Brown loam, Elk Valley, South Marin County	12	1.8
Sierra Nevada Mountain foothills			
82	Auburn red clay, west of Placerville	8	2.6
85	Aiken red clay, north east of Placerville	12	0.8
90	Rocklin brown clay loam, 10 miles east of Lodi	15	0.5
Lower San Joaquin Valley farming country			
78	Sacramento clay, 2 miles west of Sacramento	12	3.5
91	Hanford loam with sand, 3 miles east of Lodi	8	2.7
92	Greenfield brown loam, 1 mile south of Lodi	8	5.6
93	Stockton black adobe, 2 miles east of Stockton	24	2.6
94	Brentwood brown silt loam, 2 miles east of Mt. Diablo	8	1.6
Peat from Lower San Joaquin Valley			
25	Peat, Victoria Island, San Joaquin delta	12	4.0
26	Peat, Bacon Island, San Joaquin delta	12	6.2
96	Peat near Middle River, Borden Highway	8	5.4

to zinc after the zinc has been separated and estimated alone. This amount is given in the tables of analysis under X. The primary purpose of this study was to estimate available zinc in soils, therefore a reliable determination of the amounts of the other minor metals has not been made on most of the samples.

RESULTS

Representative analytical results are presented in tables 1 to 6, arranged to give a comprehensive notion of the samples as a whole, but not of nearly all the 140 samples analyzed. Figures given represent parts per million in the

air-dry material, moisture being probably less than 5 per cent in most samples. Presence of a metal not quantitatively determined is indicated in the tables by plus signs (+). Lack of information is indicated by leaders (...).

Table 1 is a summary of the complete analyses. Although more detailed descriptions with analyses of all the samples are available, this table is intended to convey some notion of the localities from which samples were obtained and of the high and low extremes of zinc found in them.

Table 2 shows the effect of long-persistent vegetation on the zinc content of the humus layer and of the topsoil. All these samples show accumulation of zinc as well as other metals in the largely organic surface soil. In samples 121 and 122, over which was a layer of several inches of decayed leaves, it appears that the zinc accumulated in the surface soil has been leached down to 10 or more inches. The inorganic soil in that locality may have as much as 5 p.p.m. of available zinc. Samples 129 and 131 were taken about 300 feet apart from the same soil type. The effect of vegetation in accumulating zinc is very pronounced in 129, though but little in 131.

Table 3 shows the relation between parent rock and resultant soil in contact with the rocks. In pairs 50 and 54 and 86 and 87, the soil was in contact with massive rock exposed above the soil. But with the other pairs of rock and soil, the soil was collected from among much broken rock mixed with the soil over areas of several acres.

Table 4 shows the great differences between different rocks in respect to their content of some minor metals.

Table 5 permits comparison of the zinc content of soils with their content of other minor metals.

Table 6 presents some results on the zinc content of a few agricultural soils.

DISCUSSION

Exercise of the ordinary care expected in average analytical work is sufficient to reproduce results within 1 p.p.m. for Zn, Cu, and Pb. For Co and Ni less accurate results may be obtained. Since the accurate determination of these metals was not considered important for this investigation, no great effort was made for higher precision. Many duplicate determinations of zinc agree within less than 1 p.p.m., though on samples high in zinc greater differences are found. For practical purposes the accuracy is ample.

In considering the significance of this work, it should be remembered that the purpose has been to estimate, not the total quantities of these elements in the soil, but only the amounts which might be expected to become available to plants through the medium of a soil solution. The total of these metals present in soil is usually much higher than the so-called available.

Reckoned as percentage of the whole soil, the amount of zinc as well as of the other minor metals is very small, but greatly varied. In soils that may be considered tillable the variations are very much less than in soils of rough virgin land. The higher amounts are found in rock samples or in soils containing much organic matter which has accumulated on the surface as fallen leaves and

other vegetable debris where the ground has been long undisturbed (see table 2). It is assumed that the concentration of zinc, and in some instances of other metals, in the humus layer on top of some soils is caused by action of the plants which absorb it from the soil then let it fall back to the surface as dead leaves. When this vegetable matter decays, the zinc is quickly fixed in water-insoluble state by the surface soil so that it is no longer accessible to the main root systems of the plants unless the humus layer is mixed with the soil by tillage or other disturbance.²

Less zinc is usually found in the subsoil than in the surface, as is shown by samples 1-16 (table 1) and others.

Because of the effect of vegetation in accumulating organic matter containing zinc on the surface of the soil, great variations in zinc content in respect to depth of sample are common. This is apparently the reason for lower zinc content of the subsoil where samples were taken from topsoil and subsoil in the same place. Large variations in zinc in samples taken from essentially the same spot at different times are probably also due to the large variation in amount of decayed vegetable matter. This emphasizes the importance of recording, at the time of sampling, the exact depth from which the sample is taken, with an estimate of the amount of organic matter collected on the soil. Also it indicates that a representative sample of any locality should be composed of a thorough mixture of numerous subsamples from spots judiciously selected to represent the whole area sampled.

Large variations in the amount of lead, copper, cobalt, and nickel in relation to zinc are found in some samples, as set out in table 5. Some of these, as the nickel and lead in 38, may be due to accidental contamination of the soil by human agency, but in most cases it seems more likely that such variations are due to a different kind of rock from which the soil developed. In the figures indicating composition of some rock samples which differ widely from average soil samples (table 4), an unusual case is 114, a decomposing shale. This was divided into yellow and brown layers separated only a few millimeters from each other in the rock. Yet the two layers contained very unequal proportions of zinc, while 115, from nearby, had much larger amounts of zinc. The zinc in peat samples 25, 26, and 96 (table 6) is not high if it is calculated on the volume instead of the weight of the soil.

Many samples were collected for the purpose of finding out whether soil and rock from the same place were nearly alike in composition (table 3). In most of these cases the rock appeared to be the parent source of the soil, and in such instances, the two were similar in composition. In other cases, however, there is wide variation between rock and soil. Possibly, individual pieces of loose rock found on the surface of the soil may have been brought there by some agency in no way connected with the development of the soil, and therefore no great similarity in composition should be expected.

² After this had been written it was found that a similar hypothesis had been offered by Goldschmidt (2) to explain how various metals are sometimes accumulated by the agency of vegetation on the surface of the soil.

On account of the great capacity of the soil to convert zinc to a water-insoluble condition it appears improbable that zinc brought to the surface of the soil by plant action will soon be leached down into the subsoil by ordinary weathering agencies. But the fact that the zinc content of many soils is similar in samples which have been taken from widely separated places and which have been developed from very different kinds of rocks, seems to indicate that the soil-forming agencies tend to affect the distribution of zinc in about the same way as they affect the distribution of other difficultly soluble mineral matter in soil. This is in accord with the thesis that similar weathering tends to produce similar soils from widely different sources. This tendency to uniformity in soils with respect to zinc will be promoted by the presence of soluble salts and CO_2 in the soil solution, because these greatly increase the solubility of zinc over its solubility in rain water.

During the study of methods for extracting zinc from soils, it was found that little or no zinc was removed from the soil by alkaline solvents. Failure of some plants on alkaline soils may be due to lack of available zinc in the soil solution, though the potential supply is adequate. Perhaps this is the reason why fruit trees sometimes fail on so-called corral spots, spots where animals have been corralled, frequently for many years in succession. The soil is generally alkaline, and contains unusually large amounts of easily soluble potassium and phosphate. The alkalinity and the phosphates are unfavorable to solubility of zinc. It may be that acidification of such soils would make them more fertile. Perhaps the recovery of fruit trees on such soils following growth of alfalfa among the trees may be aided by removal of excessive phosphate and increase of CO_2 in the soil through action of the alfalfa.

A general survey of the analytical results leads to the conclusion that there are large differences in the zinc content of soils from different locations, sometimes from spots near each other, such as 129 and 130 (table 2), caused by organic matter. Percentage variations in available zinc seem to be similar to those found in available potassium, phosphorus, and other nutrient constituents of ordinary soils.

Too few agricultural soils have been examined to warrant any sweeping conclusions in regard to zinc content of soils from different regions or of different series. It appears that many more samples of soil should be analyzed to determine (a) differences in the same spot relative to depth, (b) differences in the same kind of soil in different locations, (c) whether there is a characteristic difference between different soil series, and (d) whether it is possible to predict the probable adequacy of the zinc-supplying power of a soil.

SUMMARY

About 140 samples of soil and rock collected from numerous places in central California have been analyzed for available zinc. In some samples copper, cobalt, and nickel also have been determined. Lead was determined in all samples.

In general, the amount of zinc found is only 1 to 5 p.p.m., though some samples contain much more.

Many samples show that zinc as well as some other minor metals are accumulated in the surface soil by action of vegetation, and that this accumulated zinc is only very slowly leached down because of the high fixing power of soil for zinc. Probably this is the reason why subsoil usually contains less zinc than does surface soil.

Many samples of rock associated with the soil have zinc content similar to the soil, indicating that the soil was derived from the rock. Although the zinc content of soils from different regions is sometimes very different, it seems probable that soil-forming agencies, excepting vegetation, tend to produce soils having everywhere a similar content of zinc and other minor metals.

Though the amount present may be adequate, the zinc in alkaline soils seems to be relatively unavailable to some species of plants.

REFERENCES

- (1) FURNSTAL, A. H. 1935 A simple rotating ball mill. *Indus. and Engin. Chem., Analyt. Ed.* 7: 342.
- (2) GOLDSCHMIDT, V. M. 1937 The principles of distribution of chemical elements in minerals and rocks. *Jour. Chem. Soc.* part 1: 655-673.
- (3) HIBBARD, P. L. 1938 Estimation of copper, zinc and cobalt (with nickel) in soil extracts. *Indus. and Engin. Chem., Analyt. Ed.* 10: 615-618.
- (4) HIBBARD, P. L. 1940 Chemical status of zinc in the soil with methods of analysis. *Hilgardia* 13: 1-29.
- (5) KIDSON, E. B., ASKEW, H. O., AND DIXON, K. JR. 1936 Colorimetric determination of cobalt in soils and animal organs. *New Zeal. Jour. Sci. and Technol.* 8: 601-607.
- (6) ROLLET, A. P. 1926 Test for nickel. *Chem. Abs.* 20: 3274.
- (7) VAN KLOOSTER, H. S. 1921 Nitroso R salt, a new reagent for detection of cobalt. *Jour. Amer. Chem. Soc.* 43: 746-749.

CONSISTENCY AND PHYSICOCHEMICAL DATA OF A LOESS PAMPANEO SOIL: I. PHYSICOCHEMICAL PROPERTIES OF SAMPLES FROM DIFFERENT DEPTHS OF A PROFILE

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This investigation was started to determine the surface chemical factors of a typical Argentinian soil—a loess pampaneo—and their relations to the engineering properties of this soil. The information reported here is to be used as a basis for the development of a method or methods for the improvement and stabilization of this type of Argentinian soil for road-building purposes. The total investigation will comprise fundamental physicochemical experiments on the natural soil and its clay fraction, as well as the testing of systems composed of the natural soil and of admixtures of organic and inorganic nature. The present report contains physical and chemical data and the results of consistency tests on samples from an Argentinian profile and on samples of typical topsoil and subsoil taken from the same region.

SOIL MATERIAL USED

Samples of topsoil and subsoil and a monolith of the profile of loess pampaneo were taken from the right-of-way of the road from Buenos Aires to Mar de la Plata, Etcheverry Chascomus section, Station 33294. The soil samples are typical of the soil in the northeastern part of the Province of Buenos Aires and of a great part of the Province of Sante Fe. The depth of the profile is about 40 inches.

Before shipment from Argentina, the soil was treated with sulfur dioxide vapor to kill the vermin and to sterilize the soil. Prior to entering the United States, the soil was treated by dry heat at 100° C. by the U. S. Department of Agriculture. These treatments are likely to leave their imprints on the colloidal properties of the soil in question. Thus it cannot be expected that the data reported here correspond strictly to the behavior of the soil *in situ*. It appears plausible, however, on the basis of present knowledge of soil behavior, that these treatments did not affect the soil properties much more than do the common methods of sampling, drying, and pulverizing before

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usage. Besides, the purpose of this investigation was not so much to obtain absolute data as to demonstrate functional relationships.

Visual inspection of the soil profile revealed the presence of a considerable amount of organic material, especially in the upper horizons. Since organic matter is known to exert a marked influence on the physical and surface chemical properties of soils, it was decided to pay attention to the amount and, to a certain extent, to the kind of organic matter in the various horizons of the profile.

The topsoil sample is representative of horizon A of the profile, and the subsoil sample is representative of horizon B₁ of the same profile. The usual thickness of the A horizon in its natural state varies between 12 and 15 inches.³ The B horizon of the profile is of a highly clayey character 15 to 30 inches thick. In this horizon there are generally two subhorizons, B₁ and B₂, the first being richer in colloids. At the bottom of B₂, this horizon blends with the C horizon (parent material of loess pampaneo). The soil of the B₁ horizon is dark brown and in some places has dark streaks or veins due to humus material from the A horizon. It has a granular structure when dry and has high contraction and plasticity.⁴

PROPERTIES DETERMINED AND METHODS USED

Samples from the different horizons of the profile were taken for mechanical analysis and for determination of Atterberg consistency constants, type and amount of organic matter, sorption of water and gasoline, and heat of wetting in water and benzene. Samples from the topsoil and the subsoil were also taken for mechanical analysis and for determinations of Atterberg consistency constants, organic matter, sorption of water and carbon tetrachloride, and heat of wetting. Tests were also made on the profile, topsoil, and subsoil samples for the following properties: volume change at field moisture equivalent, shrinkage limit, shrinkage ratio, field moisture equivalent, and moisture equivalent (vacuum).

The mechanical analyses, by the Bouyoucos method, and the consistency tests, by standard procedures, were made at the materials laboratory of the Missouri State Highway Department.

The sorption data were obtained with the Winterkorn-Baver sorption apparatus (5).

The heat of wetting data were obtained with a water calorimeter consisting of a silvered 50-cc. Dewar flask fitted with a three-holed cork in which were inserted a thermometer, an electric stirrer, and a glass tube inlet for the soil. For each determination, 20 cc. of water and 1 gm. of soil were used. The water value of the calorimeter was 12.6 gm. With the help of a magnifying glass the

³ In most cases these soils belong to group A₄ of subgrades according to the classification of the Bureau of Public Roads.

⁴ According to the classification of the Bureau of Public Roads, it belongs to group A₆ or A₇.

thermometer could be read accurately to 0.05° C. and approximately to 0.02° C.

The amount of organic matter was determined by combustion; and its nitrogen content, by the Kjeldahl method.

DISCUSSION OF THE DATA OBTAINED

The results of the routine tests are contained in table 1; the data on sorption, heat of wetting, and organic matter are shown in figures 2-5. These are discussed in the following paragraphs.

TABLE 1
Routine tests on soil samples

LAYER (INDEX)	LOWER LIQUID LIMIT*	LOWER PLASTIC LIMIT*	PLASTIC INDEX	VOLUME CHANGE AT FIELD MOISTURE EQUIVALENT*	SHRINKAGE LIMIT*	SHRINKAGE RATIO*	FIELD MOISTURE EQUIVALENT*	MOISTURE EQUIVA- LENT (VACUUM)*	MECHANICAL ANALYSIS†			
									Pass- ing # 200 sieve	Silt (Diam. 0.05- 0.005 mm.)	Clay (Diam. 0.005 mm.)	Col- loid. (Diam. 0.001 mm.)
<i>inches</i>												
<i>Profile samples</i>												
0-2	38.5	31.9	6.6	11.9	27.1	1.38	35.7	30.3	95.0	53.0	26.0	11.0
2-7	33.5	26.1	7.4	10.8	22.8	1.54	29.8	28.5	95.8	53.0	26.0	11.0
7-10	33.3	16.4	16.9	9.5	18.0	1.73	23.5	31.7	95.4	47.0	34.0	20.0
10-15	50.0	21.6	28.4	29.0	15.2	1.86	30.8	44.4	97.0	37.0	49.0	32.0
15-24	69.0	27.8	41.2	44.8	16.8	1.83	41.3	63.8	98.8	30.0	61.1	37.0
24-32	61.2	25.7	35.5	39.7	16.3	1.83	38.0	55.7	98.6	31.0	56.0	31.0
32-39	44.2	25.1	19.1	30.4	15.7	1.79	32.7	44.1	98.0	37.0	50.0	27.0
<i>Topsoil and subsoil samples</i>												
Topsoil	38.4	28.6	9.8	14.7	24.0	1.48	33.9	29.9	95.8	51.0	29.0	13.0
Subsoil	60.7	27.4	33.3	46.3	14.4	1.89	38.9	55.8	98.2	33.0	53.0	34.0

* Results on material passing #40 sieve.

† In per cent.

Change of the soil constants as a function of the location of the sample in the profile

Figure 1 shows the change of the liquid, plastic, and shrinkage limits for increasing depth. It is only from 10 inches down that these constants follow the same type of curve. Tammann (4, p. 70-72) asserts that a plastic body must have at least three glide plane systems, that the breaking strength of a crystal element bounded by three glide planes must be as great as possible compared with the force necessary for gliding, and that the greater the number of glide planes per centimeter in one and the same glide system the more plastic is the body. Accordingly the more plate, scale, or rodlike particles there are in a soil system and the smaller their dimensions, the greater is the potential plasticity of the system. To fulfill the second of Tammann's requirements for plasticity, the surfaces of the mineral units of the soil have to

be more or less well lubricated. The more surface there is in a soil system, the more lubricant is required; accordingly the higher the clay and colloid content, the higher should, in general, be the plastic limit; on the other hand, the increase of the number of sliding planes with increasing amount of fines should have a tendency to lower the plastic limit values; thus, the relationship between plastic limit and clay or colloid content would not be expressible by a straight line. Another influence in the same direction is the sorption of clay particles on larger grains such as those of silt and sand, exempting one side of the clay particles from the need of lubrication. By taking these factors into account it can be seen, even if we assume that all the fine soil particles possess the same

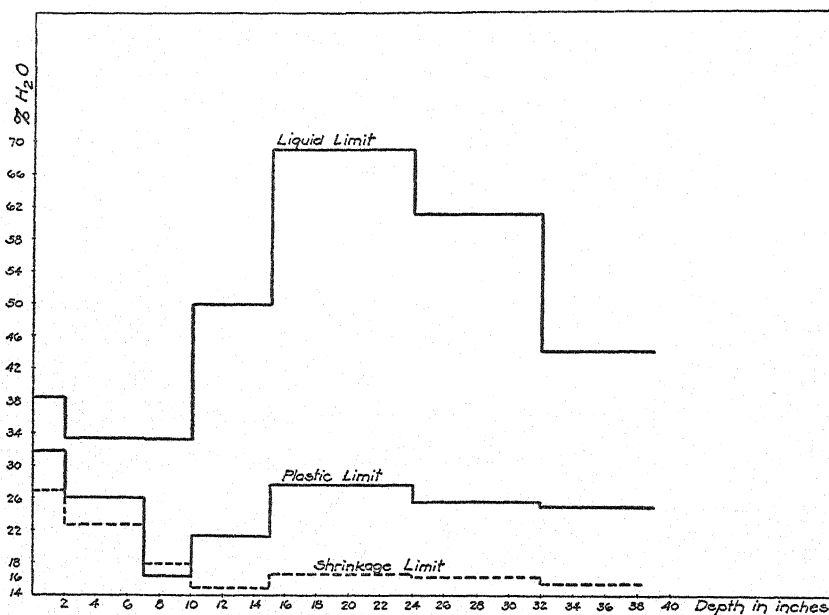


FIG. 1. CHANGE OF WATER-CONSISTENCY RELATIONSHIP WITH INCREASING DEPTH IN PROFILE

surface character and the same lubrication needs, that a strict proportionality between clay and colloid content on one side and the plastic limit on the other cannot be expected. Actually the surface and lubrication behavior of the fine materials in soils may differ not only for different geographic locations, but for different depths on the same location caused by differences in their chemical composition, in their exchange ions, and in their surface coatings. Thus the deviation of the curve type of the plastic limit function from that for the clay and colloid function may be caused by one or more of the aforementioned factors. Though the water at the plastic limits represents the more strongly bound lubrication liquid, the difference between the water content at the plastic and the liquid limits represents water bound with less and less energy and grading

into free water at the liquid limit. Between these limits thixotropic phenomena may play a considerable role.

Although the contents of clay and colloids were constant from the surface to a depth of about 7 inches, there was a considerable difference between the data on the liquid limit, the plastic limit, and the shrinkage limit at depths of 0-2 and 2-7 inches, respectively. Although the clay and colloid contents at a depth of 2-7 inches differed considerably from those at a depth of 7-10 inches, this difference appeared only in the plastic and shrinkage limits, and not in the liquid limits. At the same interval, the value for the shrinkage limit, which commonly lies lower than that for the plastic limit, is above the

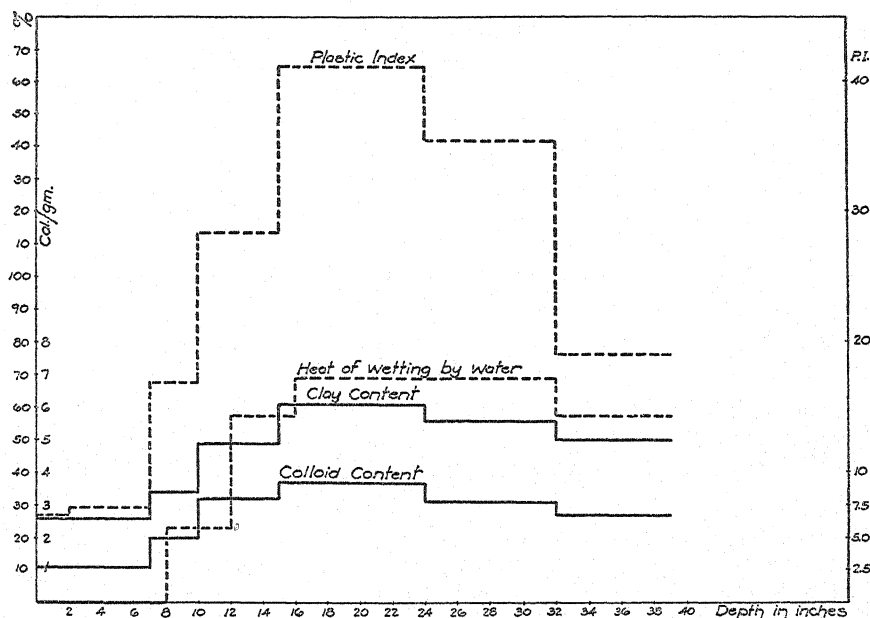


FIG. 2. CHANGE OF SOIL PROPERTIES WITH DEPTH

latter. This phenomenon as well as the aforementioned one indicates expansive properties of the layer from 7 to 10 inches in completely wet and in dry condition, while this property is probably checked by the surface tension of water at intermediate moisture contents.

Figure 2 shows data on the plastic index, the heat of wetting by water, and the clay and colloid contents. As expected, the general type of curve for the plastic index corresponds to that for the content of clay and colloid. But between 2 and 7 inches the plastic index increases without any increase in the clay and colloid contents. Similarly, the difference in plastic index between the soil at 24-32 inches and that at 32-39 inches appears greater than that warranted by simple decrease in clay and colloids.

Energy of wetting as a function of the surface chemistry of the soil constituents

No heat of wetting could be obtained by water immersion of the soil samples taken from a depth of 0 to 8 inches. For greater depths, the heat of wetting in water rises in accordance with the clay content but stays constant for samples from a depth of 16 to 32 inches, without registering the change of clay content occurring at 24 inches. The resistance of the organically coated surface soil against wetting by water appears to be the reason that more water is needed for the lubrication of the fine particles in this soil and for the consequent relative increase of the plastic limit. Contributive to this effect are also the expansive properties of the surface soil. The reason for the constancy of the heat of wetting for the soil samples from 16 to 32 inches can be found in the two facts, (a) that not all the potential internal surface of the soil systems was accessible to the wetting water, and (b) that the water in order to reach the surfaces to be wetted had to overcome the resistance of separation, with the loss of part of the heat of wetting. Of course, in preparation of the samples for the plastic and liquid limit tests this resistance is mechanically overcome by the operator, who works the water into the soil mass. For this reason the plastic index shows a parallelism with the clay and colloid content of the samples. This is further brought out by the data in figure 3 showing the changes in plastic index, heat of wetting in water and in benzene, respectively, and water and gasoline intake by powdered soil samples. Though with water no heat of wetting is produced on powdered soil samples taken from a depth of 0-8 inches, wetting with benzene shows a heat effect, and samples taken from depths below 8 inches are still better wetted by benzene than by water. Samples of the natural soil taken from greater depths are more easily wetted by water than by benzene. In accordance with the trend of the data on the heat of wetting in water, the data on the heat of wetting in benzene do not show a maximum at the maximum of the clay and colloid content. Neither do the data on the intake of water and gasoline by the dry powdered soil sample show this maximum. On the contrary, there exists a secondary minimum for the water intake at this place. The water intake measured by the Winterkorn-Baver method shows, first, a very slight decrease with increasing depth of sampling, then an abrupt increase, with continued tendency to show an increase. The intake of gasoline, is, as was expected, highest for the soil samples from the top 4 inches. The hidden maximum in the intake and heat of wetting data indicates that more energy might be needed to destroy the agglomerations of the fine particles than could be furnished by the energy of hydration.

In figure 4 are given curves for the change with depth of the plastic index-clay ratio, the content of organic matter, the C/N ratio of the latter, and the product organic matter \times C/N ratio. It has already been shown that the ratio of plastic index to clay content cannot be expected to be constant. It is important to find out which one of the cooperating factors is chiefly responsible for the observed phenomenon. Comparison of the curves for silt content and

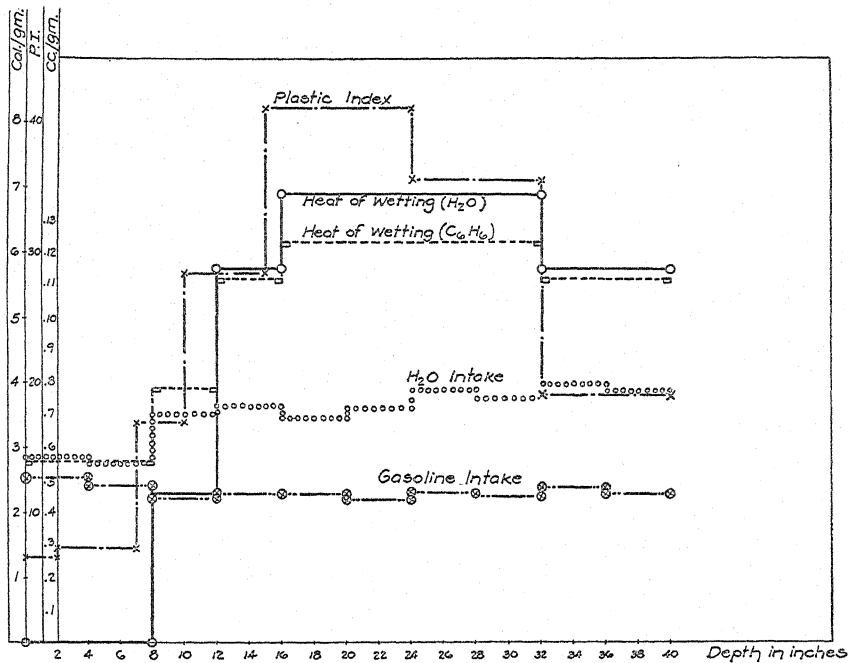


FIG. 3. RELATIONSHIP OF PLASTICITY, SWELLING, AND SURFACE ENERGY

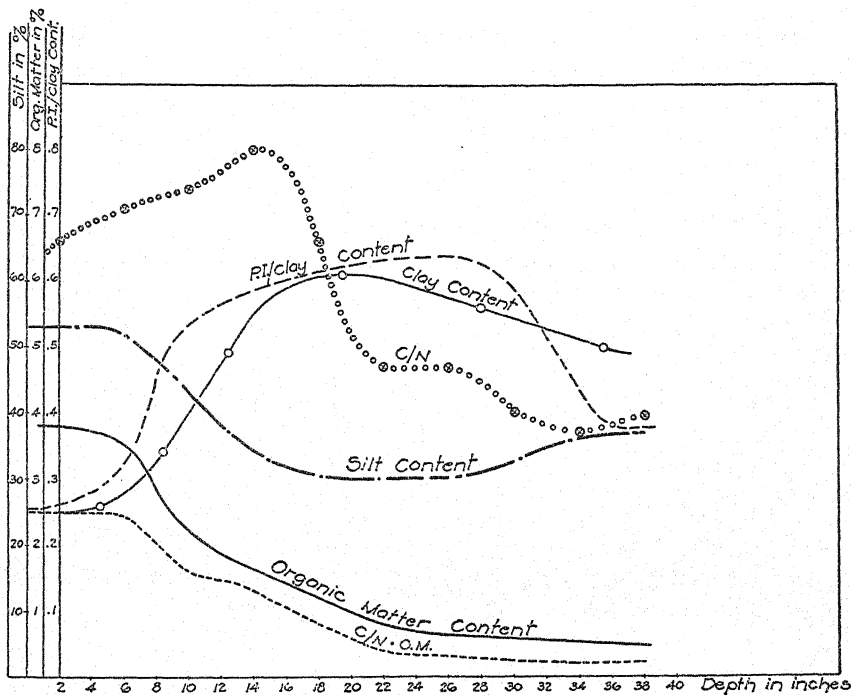


FIG. 4. FACTORS INFLUENCING THE PLASTIC INDEX VALUE

plastic index-clay ratio shows that small values of this ratio coincide with large values of the silt content; but the maximum of the plastic index-clay function does not coincide with maximum clay content. The general correlation between the plastic index-clay ratio and the silt content may find its explanation in the absorption of part of the clay by the silt particles; the larger the silt content, the larger the absorbed part of the clay, the smaller is the relative activity of the clay. This explanation appears to be the most plausible for the present case. Of course, there are other possibilities, as already noted. Thus the clay may vary in chemical composition and therewith in physical properties with increasing depth in the profile. The clay may also exist in different states of aggregation and reactivity for different depths as a result of (a) the amount, and (b) the chemical composition and physical properties of organic coatings. There is, furthermore, a possibility that the effect of organic cements falsifies the data of the mechanical analysis. Compared with the effect of the silt content, the other possible effects appear to play only a secondary role in the present case for the lower parts of the profile. The data plotted in figure 4 lead to interesting conjectures on the relative participation of the enumerated factors on the plastic index values, but it would be premature to attempt quantitative statements in view of the scarcity of material on hand.

Influence of organic matter on the consistency properties of soils

A point of major interest is the influence of the organic matter on the consistency properties of soils. Russel and Wehr (3) found that the organic matter has a decided effect on the plasticity of soils; Burr and Russel (2) showed that the loss of organic matter decreased the plastic range and increased the toughness of soils. Bayer (1) oxidized soils containing organic matter with a 3 per cent H_2O_2 solution and found a marked lowering of both the plastic and the liquid limits of the soils after this treatment, though the plastic index showed only a slight tendency to decrease. These findings are in accordance with the data contained in this paper, but it must be kept in mind that the oxidation of the organic matter might be accompanied by an oxidation of the inorganic surface constituents of the soil particles with some modification of their activity toward water. It is probable, furthermore, that extended drying is likely to change the affinity of the organic material for water. Another interesting finding of Bayer's is the increase of the amount of clay and the decrease of the amount of silt and sand in soil samples by oxidation. This fact confirms the statements on the possible function of organic matter which were made in the foregoing.

It appeared worthwhile to find out how oxidation of the soil samples from different depths of the profile influenced the heat of wetting with water and benzene and the intake of water and gasoline in the Winterkorn-Bayer apparatus. The samples were oxidized with 10 per cent H_2O_2 ; the results are seen in figure 5.

The data show, in general, a greater heat of wetting for benzene than for

water. The heat of wetting for benzene is constant to a depth of about 8 inches, then increases rapidly to a maximum at about 18 inches (coinciding with that for the clay content), decreases for samples taken from depths down to 26 inches, and continues constant. Except for a small part (24–28 inches) the curve for the heat of wetting with H_2O lies beneath that obtained with benzene. The heat of wetting with H_2O per gram of soil is constant to a depth of about 12 inches, rises rapidly to a depth of 18 inches, stays constant to a depth of about 26 inches, falls to a depth of 30 inches, and remains constant to the end of the profile. There is no maximum which coincides with that of the clay content, as was the case with benzene. In soil samples from the upper horizon some organic matter appears to be available, which cements some of the clay

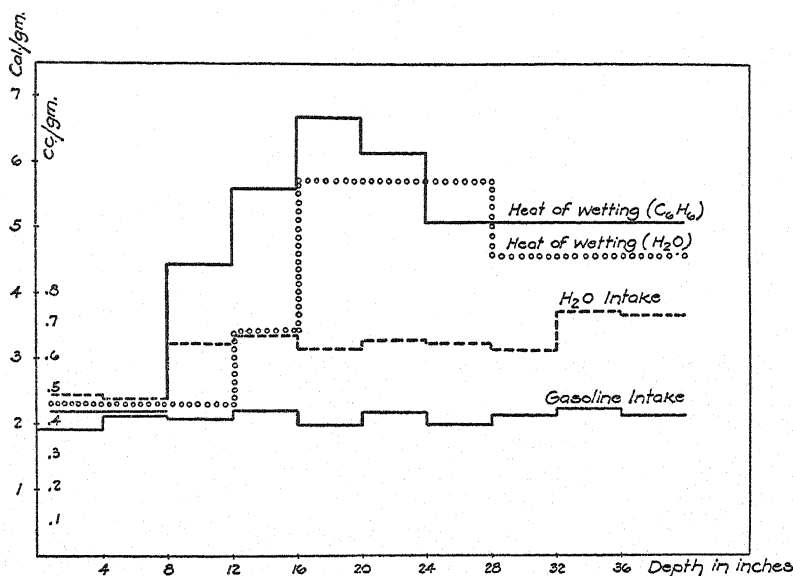


FIG. 5. HEAT OF WETTING AND SORPTION DATA OF OXIDIZED SOIL

particles together and which has a greater affinity for benzene than for water. The constancy of the heat of wetting for water from 18 to 26 inches seems to indicate that the energy of cementing the clay particles together is at least of the same order of magnitude as the energy of the wetting process. The water sorption stays constant to a depth of about 8 inches, increases rapidly with increasing depth to about 12 inches, after which it stays more or less constant to a depth of about 32 inches. A rapid increase at this point coincides with an increase in silt content. The intake of gasoline is slightly irregular but has a tendency to stay constant. These data demonstrate, again, that the behavior of soil toward water is not just a simple function of the primary constituents of the soil such as sand, silt, and clay, as determined by mechanical analysis, but is greatly influenced by the secondary structure and by the stability of this

secondary structure. The stability of the secondary structure decides to a large extent the difference in the results of test methods which involve manipulation and of those which do not. It is obvious that the manipulation involved in testing a soil for a certain purpose should not be more intensive than the manipulation which the soil has to stand in service. Thus in subsoils it is not the potential water capacity, as expressed by the liquid limit, the plastic limit, and the plastic index, which is important in judging their qualities, but rather their reaction toward the water that actually enters the system by such forces as capillarity, and the resistance of the secondary particles toward dispersion by the water alone or in connection with stresses and strains incidental to their usage.

SUMMARY AND GENERAL CONCLUSIONS

Data on the mechanical composition, consistency properties, heat of wetting, and sorption of liquids were obtained with soil samples taken from different depths of a profile of loess pampaneo. They were used as a basis for discussing the various factors which influence the soil-water relationship. Among others, the following facts were determined:

A large clay content and a large plastic index indicate rather the potential water-holding capacity of a soil than the behavior of the natural soil toward the action of water *in situ*.

The actual behavior of a relatively dry soil system toward water can be represented as a dynamic equilibrium between the wetting energy of the water and the cohesive forces at play in the soil system. The mechanism of the water attack is a function of the accessibility of the internal surface of the soil. Thus the water sorption in the Winterkorn-Baver apparatus appears to be more dependent on wetting phenomena and on the amount of silt present than on the total clay content.

The amount and type of the organic material in a soil exerts a considerable influence on the behavior of the soil toward water. In this connection, the change of the C/N ratio of the organic material with change in depth of the profile is probably of importance.

From a practical standpoint, soil from the surface 8 inches should make a better subgrade for roads than the subsoil. It should also give better results in bituminous stabilization, as indicated by its preferential wetting with benzene.

REFERENCES

- (1) BAVER, L. D. 1930 The effect of organic matter upon several physical properties of soils. *Jour. Amer. Soc. Agron.* 22: 703-708.
- (2) BURR, W. W., AND RUSSEL, J. C. 1927 The importance of organic matter in soil structure and tilth. *Abs. Proc. First Internatl. Cong. Soil Sci., First Comm.* 1927: 69.
- (3) RUSSEL, J. C., AND WEHR, F. M. 1928 The Atterberg consistency constants. *Jour. Amer. Soc. Agron.* 20: 354-373.
- (4) TAMMANN, G. 1925 A Textbook of Metallography. New York.
- (5) WINTERKORN, H. F., AND BAVER, L. D. 1934 Sorption of liquids by soil colloids: I. Liquid intake and swelling by soil colloidal materials. *Soil Sci.* 38: 291-398.

AUTOLYSIS OF A THERMOPHILIC ACTINOMYCES¹

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The phenomenon of autolysis or self-dissolution has been noted by many investigators working with yeasts, filamentous fungi, and many species of bacteria (1, 2, 5, 6, 9). More recently, Verner and Altergot (7) reported the lysis of mycelium of *Fusarium nivium*. Dissolution was especially marked when the organism was cultured on certain media and when its growth was accelerated. A thermostable lytic principle was isolated from the lysing cultures. Concurrently, Krasilnikov (3) described a somewhat analogous phenomenon among saprophytic and pathogenic actinomycetes. Partial or complete lysis of colonies, beginning most frequently at the center and passing into the periphery, was observed. During lysis some threads of mycelium became differentiated into chlamydospores or strongly inflated cells which did not lyse but later developed into mycelia. Following up this work, Krasilnikov and Korenieko (4) obtained a thermostable lytic factor from solutions in which lysis had occurred. The lytic agent dissolved dead cells and was strictly species specific, two factors which were considered by the investigators to exclude bacteriophage and lysozyme respectively.

When soils or decomposing materials are plated, large numbers of actinomycete colonies develop. Some of these show evidence of lytic degeneration which is usually restricted to segments of the colonies and is rarely complete. It is a common phenomenon, to which surprisingly little attention has been paid.

The present paper deals with the lysis of a saprophytic, thermophilic *Actinomyces*. During an examination of starch-ammonium-sulfate-agar plates of horse manure composted at 50°C. (8) attention was drawn to an *Actinomyces* colony which had disappeared almost completely over night. Remnants of this colony were transferred to fresh agar medium, and growth was obtained within 24 hours. Some 12-24 hours later this growth had again vanished almost entirely; further studies were therefore begun and are here briefly reported.

EXPERIMENTAL

Cultivation of the Actinomyces

An attempt was first made to obtain a medium on which the organism could be readily cultured without its undergoing lysis so completely as to

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TABLE 1
Growth and lysis of a thermophilic Actinomyces on different media

Incubation...hours	EXTENT AND CHARACTER OF GROWTH				DEGREE OF LYSIS			
	24	48	72	96	24	48	72	96
Nutrient agar	Good growth, slight development of white aerial mycelium	Extensive development of white aerial mycelium	Unchanged	Unchanged	Little or no lysis			
Czapek's agar	None	Sparse, thin development	Slight development of dull-gray white mycelium	Unchanged	Little or no lysis			
Sodium-albuminate agar	None	Sparse, thin development	Slight development of dull-gray white mycelium	Unchanged	Little or no lysis			
Dextrose-asparagine agar	None	Sparse, thin development	Sparse growth, white surface mycelium	Unchanged	Little or no lysis			
Starch-annominum-sulfate agar	Rapid growth with production of white mycelium	Extensive development of white mycelium	Lysis in localized areas on plates	Increased areas of lysis	Almost complete lysis of all growth, some mycelial debris still present	Unchanged
Potato slants	None	None	None	None

obviate all possibility of its recovery. Accordingly, remnants of growth from plates on which lysis was first demonstrated were streaked on different media (table 1) and the cultures incubated at 50°C. The medium most suitable for the cultivation of this organism was nutrient agar. This substrate in no way interfered with the lysis of the organism when transferred to an appropriate medium (starch-ammonium-sulfate agar).

Demonstration of lysis

The procedure employed in demonstrating lysis was to prepare a water suspension of a 24-48-hour nutrient-agar culture and to spread an aliquot of the suspension on the surface of the starch-agar medium in petri plates; or to transfer a suitable quantity (0.5-1 cc.) of the suspension to sterile plates into which the agar medium is poured. By this means, the gross aspects of lysis presented in figure 1 were obtained. Several of the circular lytic areas were magnified and photographed (fig. 2). These did not change in size or shape but gradually merged into, and disappeared along with, the adjacent mycelium. These clear lytic spots were remarkably similar to bacteriophage plaques although, as will be pointed out subsequently, no transmissible lytic agent could be demonstrated. Dissolution or lysis was usually complete within 48-72 hours. Nevertheless at times, clumps of mycelium remained, but when these were streaked on a suitable medium they produced growth that later again underwent lysis, which was usually but not always complete.

By means of streaking a diluted suspension of the *Actinomyces* on starch agar, colonies were obtained which began to lyse after 36 hours at 50°C. (figs. 3 and 4). Lysis may begin at the periphery and extend across the entire colony, or it may begin at the center and pass gradually to the edge. In some cases the colony as a whole slowly disappeared. At times, a slight residue of fragmented mycelia remained, but more often the colonies lysed completely.

An attempt was made to study in greater detail the disintegration of these colonies. Figures 5 to 7 illustrate the progressive lytic degeneration of one colony photographed first when 36 hours old and when lysis had already begun. The colony, originally composed of densely interwoven mycelial strands (fig. 4), gave evidence of lysis by the gradual thinning out of this mycelial mass, as may be seen in figure 3 where the centers of several complete colonies have become spotted. This diminution of density is clear in figure 5. The mycelium began to shred and break up into irregular thin threads, and finally it became more or less translucent, leaving, in many places, a difficultly discernible fine-grained residue (figs. 6 and 7).

The presence of plaquelike spots in a confluent mass of the *Actinomyces* suggested bacteriophage as the agent responsible for the lytic degeneration observed. Phages active against actinomycetes have been reported (8). No transmissible lytic agent could be demonstrated. During this work it was found that the organism grew slowly in starch-ammonium-sulfate fluid medium

with the production of fluffy lenslike colonies scattered throughout the medium. After 72 hours' incubation these disintegrated but did not lyse completely, as transfers from these cultures into fresh medium produced similar colonies followed by disintegration. Berkfeld filtrates of such fluid cultures and filtrates of extracts made with the same medium triturated with agar in which lysis had occurred were tested against the lytic *Actinomyces* and twelve common soil actinomycetes (*Act. scabies*, *Act. cellulosa*, etc.), but in no case was growth inhibited or transmissible lysis obtained. No lysis of heated or unheated suspensions of the *Actinomyces* in distilled water or of heated suspensions in the starch medium could be demonstrated.

Effect of temperature and reaction on growth and autolysis

Nutrient and starch-agar media were used in these experiments. The organism did not grow at 28°C. or 60°C.; growth was best at 50°C. and slower at 37°C. Lysis (on the starch agar) was most complete at 50°C. and was correspondingly slower at 37°C. It was found that the optimum pH for growth was from 7.0–7.5, and once more, lysis was most complete where growth was best. There was little growth below pH 6.0 and above 8.0. In the course of this work it was noted that the reaction of the starch-ammonium-sulfate agar in which lysis had occurred had dropped from pH 7.0 to about 5.7. The reaction of such agar cultures was readjusted to pH 7.0, and the medium was sterilized and reinoculated. Growth was obtained after 24–36 hours' incubation and was followed by lysis; again, the reaction dropped to pH 5.7. Starch agar was now prepared and KNO₃ substituted for (NH₄)₂SO₄. The organism grew more slowly on this medium but did not lyse appreciably, and it was found that the pH of this medium remained at about 6.8 to 7.0. These experiments demonstrated that it was not lack of nutrients which caused inhibition of growth followed by dissolution and pointed to reaction resulting from the accumulation of the sulfate ion as the factor which suppressed growth and directly or indirectly stimulated the autolytic mechanism. Autolysis of this *Actinomyces* is not alone a matter of growth, senescence, death, and dissolution, since the organism develops well and rapidly on nutrient agar without lysing appreciably.

In order to study this effect further a buffer solution of Na₂HPO₄ and KH₂PO₄ salts in the proportions required to give a pH of 7.1 was employed, and starch-ammonium-sulfate medium (fluid and solid) was prepared by adding to this buffer solution the necessary ingredients in the correct proportion. It was found, however, that the organism would not grow in this medium. Recourse was then made to the introduction of CaCO₃ into the medium; it had been omitted previously, since the white background it produced on agar plates made observation of lysis difficult. Addition of this salt to agar plates did not counteract appreciably the acidity which developed on the plates or prevent lysis. Nevertheless, growth was more abundant, and lysis was somewhat delayed and not always complete. CaCO₃ is, of

course, relatively insoluble, and its influence is particularly negative in a solid medium. Lime (1 per cent) was therefore added to starch-ammonium-sulfate fluid medium, which was then inoculated with the *Actinomyces*. The results are presented in table 2.

Again the relation between pH and lysis is evident. Appreciable surface growth was obtained in the lime-treated medium. This was removed and transferred to starch fluid medium without lime, but it did not lyse. It may be that lysis occurs at a particular stage of growth and at a certain pH, and if the organism passes the susceptible stage while the pH is held at or near the optimum (as in the medium containing CaCO_3) it will not lyse. This

TABLE 2
Influence of reaction upon growth and lysis of a thermophilic Actinomyces

Incubation.....hours	pH VALUE					GROWTH*				LYSIS†			
	0	24	48	72	96	24	48	72	96	24	48	72	96
Flasks without CaCO_3	7.0	7.0	6.8	6.3	6.1	+	++	++	+	-	+	++	++
Flasks with CaCO_3	7.1	7.0	6.9	6.8	6.8	+	++	+++	+++	-	-	-	-

* Growth: + slight, ++ fair, +++ medium.

† Lysis: - none, + slight, ++ fair.

TABLE 3
Correlative changes of reaction and lysis of a thermophilic Actinomyces

INCUBATION hours	pH VALUE	GROWTH*	LYSIS†
12	7.0	++	-
24	6.4	+++	+
36	6.0	++	++
48	5.7	-	++++
72	5.6	-	++++

* Growth: - none, ++ fair, +++ medium.

† Lysis: - none, + slight, ++ fair, ++++ complete.

possibility was checked by the following experiment. Starch-agar plates were inoculated with the actinomycete and incubated; the plates were observed frequently for extent of growth and degree of lysis, and at each period of examination the pH of the agar of several plates was determined.

It is apparent from table 3 that lysis begins at a pH higher than 5.7 and at a reaction which still permits growth. The results of this experiment, then, lead to the interpretation that it is not the low pH (5.7) which stimulates the autolytic agent by inhibiting further growth, but that it is a certain pH (6.0-6.5) which, becoming effective when the organism is at a particular stage of its development, inhibits it and stimulates the autolytic mechanism. This

conclusion is in accord with that of Krasilnikov and Korenieko (4), who stated that "if on a developing culture, however young, the effect of some agent delaying growth is exercised, then lysis begins."

SUMMARY

A thermophilic, autolytic *Actinomyces* was isolated from composting horse manure kept at 50°C.

The organism grew rapidly (24–36 hours) and well on nutrient and starch-ammonium-sulfate agars and poorly on Czapek's, sodium-albuminate, and dextrose-asparagine agars. Autolysis occurred only on the starch agar after 24–48 hours' incubation at 50°C.

During massive plate lysis, localized clear areas appeared in the confluent mycelial growth; later, these merged into, and disappeared along with, the adjacent mycelium.

Lysis of colonies usually began either at the periphery or center and passed progressively inward or outward respectively. This dissolution was found to be due to a gradual fragmentation, shredding, and thinning out of the dense, interwoven mycelium of the colonies; in many places a difficultly discernible fine-grained residue remained.

No transmissible lytic agent could be demonstrated.

In starch-ammonium-sulfate fluid medium the *Actinomyces* grew fairly abundantly, but this growth disintegrated after 48–72 hours' incubation. Berkfeld filtrates of this lysed culture had no effect on its own growth or on that of twelve common soil actinomycetes.

The temperature optimum for growth and lysis was 50°C.; the most favorable reaction was pH 7.0–7.5 with limits of growth at pH 6.0 and 8.0.

During its growth on the starch-agar medium, the organism reduced the pH from 7.0 to 5.7. The addition of CaCO₃ to the fluid medium prevented this drop in pH and inhibited autolysis.

It was demonstrated that autolysis began before the pH of the medium had dropped below 6.0, and it was suggested that a combination of a certain pH (6.0–6.5) having been attained at a time when the organism was passing through a particular stage of its development was responsible for stimulating the autolytic mechanism.

REFERENCES

- (1) BACH, D. 1929 Evolution de l'incrémentation dans les cultures de l'*Aspergillus niger*. *Bul. Soc. Chim. Biol.* 2: 1007–1015.
- (2) BUCHANAN, R. E., AND FULMER, E. I. 1930 Physiology and Biochemistry of Bacteria, vol. 3. Williams & Wilkins Co., Baltimore.
- (3) KRASILNIKOV, N. A. 1938 The phenomenon of autolysis in Actinomycetales: I. Cultural and morphological picture of autolysis. *Microbiol. (U.S.S.R.)* 7: 720. (English summary.)
- (4) KRASILNIKOV, N. A., AND KORENIEKO, A. I. 1938 The phenomenon of autolysis in Actinomycetales: II. Influence of environmental conditions upon autolysis of

- actinomycetes and proactinomycetes. *Microbiol. (U.S.S.R.)* 7: 837. (English summary.)
- (5) RAHN, O. 1932 Physiology of Bacteria. P. Blakiston's Son & Co. Inc., Philadelphia.
 - (6) STURGES, W. S., AND RETTGER, L. F. 1922 Bacterial autolysis. *Jour. Bact.* 7: 551-577.
 - (7) VERNER, A. R., AND ALTERGOT, V. F. 1937 On the phenomenon of mycophagy. *Compt. Rend. Acad. Sci. U.S.S.R.* 15: 219-224.
 - (8) WAKSMAN, S. A., UMBREIT, W. W., AND CORDON, T. C. 1939 Thermophilic actinomycetes and fungi in soils and in composts. *Soil Sci.* 47: 37-60.
 - (9) WIERINGA, K. T., AND WIEBOLS, G. L. W. 1936 De aardappelschurft en de heterolyse der schurftparasiet. *Tijdschr. Plantenziekten* 42: 235-240.

PLATE 1

- FIG. 1. 36-hour plate culture of the *Actinomyces* undergoing lysis.
FIG. 2. Magnified ($\times 5$) circular lytic areas of figure 1.
FIG. 3. Lysis of colonies on starch-ammonium-sulfate agar after 36 hours' incubation at 50°C. $\times 2.5$.
FIG. 4. Lysis of a single colony (see figure 3). $\times 12.5$.

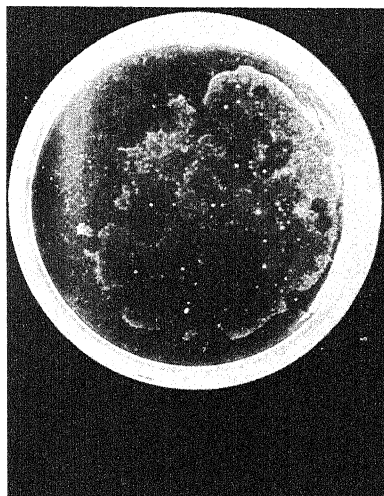


FIG. 1

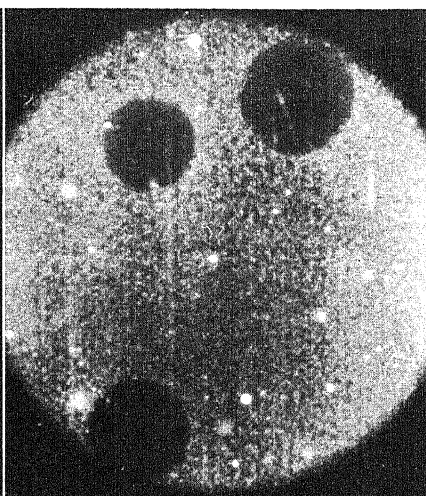


FIG. 2

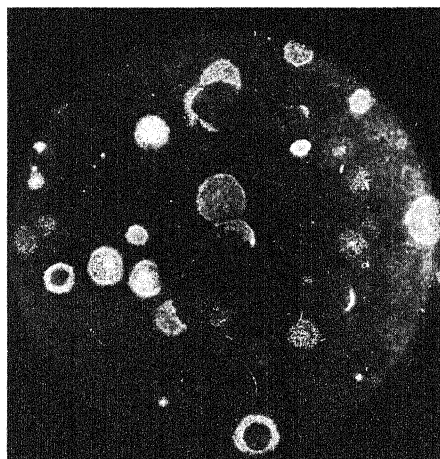


FIG. 3

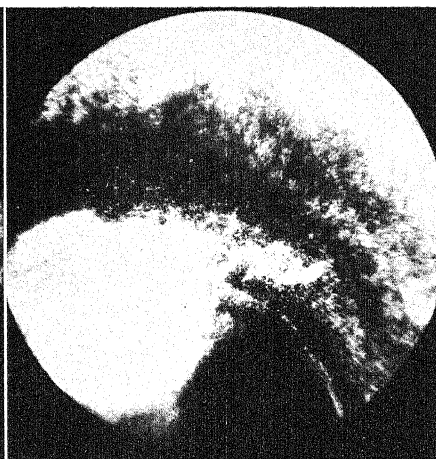


FIG. 4

PLATE 2

FIG. 5. Lysis of a 36-hour-old colony on starch agar. $\times 80$.

FIG. 6. Progressive dissolution of colony in figure 5, after 48 hours. $\times 80$.

FIG. 7. Almost complete disintegration of colony in figure 5, after 60 hours. $\times 80$.

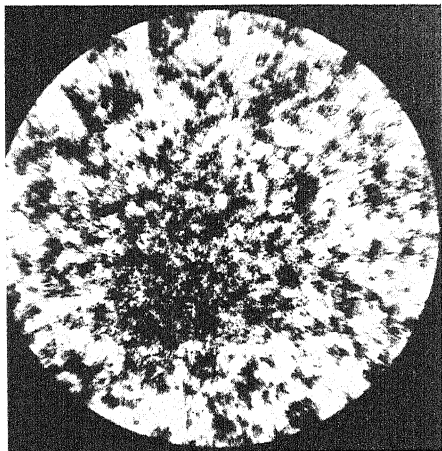


FIG. 5

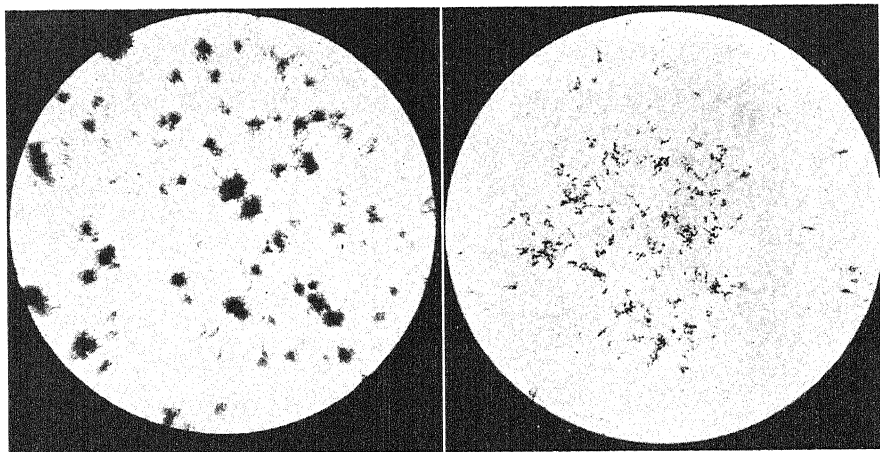


FIG. 6

FIG. 7



CHEMICAL EFFECTS OF SALINE IRRIGATION WATER ON SOILS

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When irrigation water is used for a number of years the chemical and physical properties of the soil may be markedly affected. The concentration and the composition of the salts in the water determine the speed and the nature of the changes produced in the soil.

Since irrigation waters commonly contain more or less Na salts, the importance of a thorough understanding of the base-exchange reactions which such waters are capable of producing is at once apparent. The present paper is devoted chiefly to the chemical effects of salt solutions of different total concentrations and variable ratios of bases.

EXPERIMENTAL RESULTS

The soils used in this study were: (a) Yolo clay loam from La Habra, California (No. 431); (b) Yolo silt loam from Davis, California (No. 17557), both of which are virtually free from CaCO_3 and have relatively high base-exchange capacity; (c) Dublin adobe clay from Gilroy, California (No. 7084), which contains about 1 per cent alkali earth carbonate and has very high base-exchange capacity; (d) Ramona sandy loam from Riverside, California (No. 6296), which is free from carbonate and has low base-exchange capacity.

Table 1 gives the analyses of solutions obtained by digesting 10 to 20 gm. of soil with 250 cc. neutral normal ammonium acetate for several hours at 70°C ., filtering, and leaching the sample thoroughly with ammonium acetate solution (about 500 cc.). It will be noted that the sum of the bases found in the leachates exceeded the base-exchange capacity in three of the soils. This excess, although not large with soils 431 and 17557, was undoubtedly due to soluble rather than replaceable bases in these soils. Other experiments show that a considerable part of this excess is soluble in H_2O . On the other hand, soil 7084 contains substantial amounts of substances insoluble in H_2O but soluble in ammonium acetate solution. CaCO_3 and possibly MgCO_3 comprise a part of these substances, but this soil also contains considerable noncarbonate compounds, the Mg of which is nonexchangeable but soluble in various salt solutions. For this reason, the accurate determination of the replaceable bases in this soil is extremely difficult.

Table 2 shows the effect of concentration and varying ratios of solution to soil 431. Three concentrations of chloride solution were used, each containing

Na and Ca in the ratio of approximately 2:1. The solutions were first shaken to equilibrium with the soil, then filtered and analyzed for Ca and Mg. The results show that as the ratio of solution to soil decreased, the concentrations of Ca and Mg removed in the extract increased, an effect which was partly due to soluble Ca and Mg in the soil. Originally, the solutions were free from Mg; after contact with the soil, they were found to contain substantial amounts. In every case, the solutions gained Ca over that present in the original solution. This was caused chiefly by soluble Ca rather than by replacement of Ca by Na, as will be shown more definitely in other experiments of this paper.

TABLE 1

Bases extracted from soils with ammonium acetate solution

Results in m.e. per 100 gm.

SOIL TYPE	Ca	Mg	K	Na	TOTAL	BASE- EXCHANGE CAPACITY (NH ₄ ABSORBED)
431—Yolo clay loam.....	21.70	6.00	1.36	1.22	30.28	28.30
17557—Yolo silt loam.....	11.25	15.20	1.19	1.02	28.66	23.90
7084—Dublin adobe clay.....	39.70	39.10	0.99	3.61	83.40	54.80
6296—Ramona sandy loam.....	4.60	1.34	0.21	0.66	6.81	6.80

TABLE 2

Composition of salt solutions as affected by soil 431, using various ratios of solution to soil

SOLUTION APPLIED		SOLUTION OBTAINED							
		Ratio 10:1		Ratio 5:1		Ratio 2:1		Ratio 1.5:1	
		Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
5.58	3.57	3.94	1.38	4.08	1.41	4.57	1.57	4.90	1.75
10.00	5.00	5.65	1.93	5.95	2.00	6.40	2.02	6.89	2.12
20.10	10.00	10.60	2.92	11.00	3.02	11.82	3.02	12.30	3.03

In the next experiment, 100 gm. of the same soil was leached with three successive liters of the same kinds of solutions as were used in the preceding experiment. Each liter of the leachate was analyzed for Ca, Mg, and Na. The results (table 3) show that with the most dilute solution the first liter of leachate contained more Na than the original solution. This was due to small amounts of soluble or replaceable Na in this soil. On the other hand, with every concentration used, the second and third liters of leachate contained almost exactly the same concentration of Na as the original solution. Despite the fact that considerable Mg was replaced, the first liter of leachate obtained with the more dilute solutions contained more Ca than the original solutions, an effect which, as has been mentioned, was due to soluble Ca in this soil.

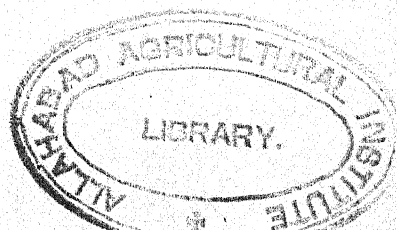
The second and third liters lost significant amounts of Ca and gained corresponding amounts of Mg as a result of base exchange.

The results of this experiment indicate that the mere application of moderately concentrated irrigation water containing two equivalents of Na, or less, to one equivalent of Ca, will not produce any important increase in the content of absorbed Na with this particular soil, but rather it will produce an actual increase in the absorbed Ca of the soil because of the replacement of Mg by Ca. Should evaporation and transpiration, however, bring about a substantial increase in the concentration of the soil moisture in excess of that of the water applied, particularly if the irrigation water is comparatively concentrated, the content of absorbed Na in the soil will tend to be increased somewhat.

TABLE 3
Composition of salt solutions as affected by leaching through soil 431

SOLUTION APPLIED			LITER OF LEACHATE	LEACHATE OBTAINED			
Na	Ca	Total		Na	Ca	Mg	Total
<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>		<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
5.58	3.57	9.15	1st	5.90	3.90	1.40	11.20
			2nd	5.53	2.88	0.81	9.22
			3rd	5.60	2.95	0.65	9.20
10.12	5.08	15.20	1st	9.92	5.37	1.91	17.20
			2nd	10.00	4.26	0.95	15.21
			3rd	10.12	4.39	0.71	15.22
20.10	10.06	30.16	1st	19.40	10.06	2.72	32.18
			2nd	19.90	8.93	1.23	30.06
			3rd	20.10	9.31	0.67	30.08

Table 4 reports the replaceable Ca and Na as affected by leaching the same soil with solutions containing Na and Ca in the ratio of 4:1. In this experiment 10 gm. of soil was leached with 2 liters of solution ranging in total concentration from about 9 to 120 m.e. per liter. This experiment undoubtedly involved more drastic leaching than is likely to be experienced in actual field practice. The results indicate that, with the less concentrated solutions, most of the exchangeable Mg originally present in the soil (5.41 m.e.) was replaced, not by Na but by Ca, as is shown by the increased content of replaceable Ca in the soil after leaching. As the concentration of the solution was increased, however, the content of absorbed Na also increased, and that of Ca decreased, the latter being roughly proportional to the increase in absorbed Na; but even after leaching with the most concentrated solution, the soil still contained more replaceable Ca than originally. This is remarkable in view of the ratio of Na:Ca in these solutions. Nevertheless, increases in replaceable Na, such as were produced by the more concentrated solutions,



would almost certainly adversely affect the physical properties of the soil to some extent (4).

Since irrigation waters commonly contain not only Cl^- but also HCO_3^- and other anions, it is of interest to compare the effects of Na salts of different anions. Table 5 shows the effects of several concentrations of NaCl vs. NaHCO_3 . One hundred grams of soil 431 was shaken to equilibrium with 500 cc. of solution, filtered, and analyzed for Ca, Mg, K, and Na. The results show that approximately the same amounts of Na were absorbed from NaCl as from NaHCO_3 , and similar amounts of Ca, Mg, and K were replaced by corresponding concentrations of both salts.

The variable pH of natural irrigation waters suggested an investigation of the effects of pH. The results recorded in table 6 show that variations in the pH of the solution ranging from 7.0 to 9.0 produced virtually no effect on the absorption of Na by soil 431. The amount of Ca found in the NaHCO_3

TABLE 4
Exchangeable Na and Ca of soil 431 as affected by leaching with salt solution containing 4 Na to 1 Ca

TOTAL CONCENTRATION OF SOLUTION APPLIED	SOIL AFTER LEACHING	
	Na	Ca
<i>m.e.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>
None	0.22	20.58
9.18	*	25.50
15.35	*	25.20
30.35	*	24.65
60.00	2.31	22.10
90.00	3.01	23.10
120.00	3.38	22.70

* Not determined.

solutions, however, tended to decrease with increased pH, probably because of the precipitation of CaCO_3 . In all cases, interaction with the soil lowered the pH of the more alkaline solutions.

It would be highly erroneous to conclude from this experiment that the absorption of Na by soil is entirely independent of pH. As was shown by Kelley and Cummins in 1921 (3), Na, in the form of Na_2CO_3 and NaOH, is absorbed by soils to a much greater extent than from corresponding concentrations of neutral Na salts. This is probably due chiefly to the fact that the solubility of Ca and Mg compounds, formed in consequence of base exchange, tends to decrease when the pH of the final solution exceeds 8.0.

In the next experiment the ratio of Na to Ca was varied by using solutions of constant Ca and constant pH but variable Na content. Five hundred cubic centimeters of solution was shaken to equilibrium with 100 gm. of soil 431. As shown in table 7, the wider the ratio of Na to Ca in the solution, the greater

was the amount of Na absorbed by the soil and the greater was the amount of Mg replaced. With solutions containing Na and Ca in the ratio of 1:1, a small amount of Ca was also absorbed, but the 4:1 solutions gained Ca by contact with the soil, probably chiefly because of soluble Ca compounds in this soil.

TABLE 5
Effect of concentration of Na salt solution on soil 431

SOLUTION APPLIED		SOLUTION OBTAINED				Na ABSORBED
		Ca	Mg	K	Na	
	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
NaCl	2.6	1.10	0.54	0.44	2.01	0.59
	5.2	1.60	0.54	0.50	3.80	1.40
	10.4	2.22	1.13	0.56	7.74	2.66
	20.8	3.63	1.70	0.56	15.40	5.40
	41.6	5.75	2.64	0.87	34.60	7.00
NaHCO ₃	2.6	0.60	0.68	0.34	1.86	0.74
	5.2	0.95	0.54	0.41	3.54	1.66
	10.4	1.95	1.10	0.51	7.70	2.70
	20.8	3.13	1.34	0.55	15.50	5.30
	41.6	5.25	2.24	0.64	34.10	7.50

TABLE 6
Effect of pH of salt solutions on soil 431

SOLUTION APPLIED	pH OF ORIGINAL SOLUTION	SOLUTION OBTAINED						Na ABSORBED
		pH	Ca	Mg	K	Na	Total	
			<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
NaCl 20.8 m.e.	7.0	7.4	3.63	1.70	0.56	15.40	21.29	5.4
	7.5	7.4	3.60	1.65	0.60	15.80	21.65	5.0
	8.0	7.6	3.55	1.44	0.61	15.60	21.20	5.2
	8.5	7.9	3.58	1.53	0.61	15.00	20.72	5.8
	9.0	8.0	3.20	1.43	0.58	16.00	21.21	4.8
NaHCO ₃ 20.8 m.e.	7.0	7.4	3.13	1.34	0.55	15.50	20.52	5.3
	7.5	7.5	2.75	1.43	0.56	15.60	20.34	5.2
	8.0	7.7	2.63	1.40	0.54	15.30	19.87	5.5
	9.0	7.8	2.10	1.36	0.54	15.70	19.70	5.1

Since the irrigated soils of the Western States commonly contain CaCO₃, a study was made on the effect produced by adding CaCO₃ to soil 431. Table 8 shows that the absorption of Na by this soil was definitely diminished by the presence of CaCO₃. From this experiment it is reasonable to conclude that, other conditions being equal, the absorption of Na from irrigation water

will be substantially less when applied to calcareous soil than to noncalcareous soil.

The data of table 8 have a bearing on laboratory methods for the determination of the replaceable bases of soils and also on the interpretation of laboratory data on the effects of saline solutions. The mere addition of CaCO_3 not only repressed the absorption of Na, but also produced an increase in the

TABLE 7
Effect of solutions of various Na:Ca ratios on soil 431

SOLUTION APPLIED				SOLUTION OBTAINED					Na ABSORBED
NaCl	NaHCO_3	CaCl_2	Ratio Na:Ca	Ca	Mg	K	Na	Total bases	
m.e.	m.e.	m.e.		m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
10.4	0	10.4	1:1	9.30	2.98	0.85	9.10	22.23	1.30
20.8	0	10.4	2:1	10.70	3.40	0.89	18.30	33.29	2.50
41.6	0	10.4	4:1	12.45	3.80	0.96	36.54	53.75	5.06
0	10.4	10.4	1:1	8.82	2.72	0.75	8.90	21.19	1.50
0	20.8	10.4	2:1	8.70	2.92	0.83	17.61	30.06	3.19
0	41.6	10.4	4:1	10.95	3.26	0.90	35.55	50.66	6.05

TABLE 8
Effect of CaCO_3 on bases extracted from soil 431 with salt solutions

SOLUTION APPLIED			ADDITIONS TO SOIL	SOLUTION OBTAINED					Na AB- SORBED
NaCl	NaHCO_3	CaCl_2		Ca	Mg	K	Na	Total bases	
m.e.	m.e.	m.e.		m.e.	m.e.	m.e.	m.e.	m.e.	m.e.
20.8	0	0	None CaCO_3	3.63	1.70	0.56	15.40	21.29	5.40
				5.43	2.03	0.60	18.30	26.36	2.50
0	20.8	0	None CaCO_3	3.13	1.34	0.55	15.50	20.52	5.30
				4.08	1.90	0.54	18.20	24.72	2.60
10.4	10.4	0	None CaCO_3	3.43	1.68	0.58	15.60	21.29	5.20
				4.55	1.87	0.56	18.10	25.08	2.70
10.4	0	10.4	None CaCO_3	9.30	2.98	0.85	9.11	22.24	1.29
				9.25	2.85	0.85	9.68	22.63	0.72

Ca extracted and to a less extent in the Mg also. As is well known, CaCO_3 is soluble to some extent in neutral solutions of NaCl and other Na salts. The Ca ions thus brought into solution tended to replace Mg, as well as to repress the absorption of Na. Where the solution contained equal concentrations of both NaCl and CaCl_2 , however, the solubility of CaCO_3 was diminished, and consequently the additions of CaCO_3 produced but little effect on the absorption of Na.

These results are in agreement with the conclusions previously drawn from this laboratory, namely, that the mere determination of the amount of bases brought into solution by treating a soil with a salt solution does not accurately measure the base exchange which has taken place. Soil scientists have frequently disregarded this fact (2). In the interpretation of experimental results on this subject, consideration must be given to the absorption of ions from the solution, as well as to the release of ions to the solution. Where the object is to determine the absorption of Na by soils containing CaCO_3 and other constituents common to many semiarid soils, the mere determination of the total Ca or other bases released from the soil may lead to highly erroneous conclusions regarding base exchange. The equilibria involved when saline solutions are added to heterogeneous mixtures of materials, like semiarid soils, are extremely complex and involve both ion exchange and solution and decomposition processes, and with some soils the amount of the latter may equal or exceed that of the former.

The foregoing experiments were made with a Yolo soil relatively high in replaceable Ca, intermediate in Mg, and moderately high in base-exchange capacity. In the following experiment, the effect of concentration of NaCl was studied on two soils (Nos. 17557 and 7084) high in Mg, one of which (No. 7084) has a very high base-exchange capacity, and on a third soil (No. 6296) low in base-exchange capacity and low in exchangeable and soluble Mg. The results show that the absorption of Na from the more dilute solutions of NaCl was approximately the same with Yolo soil 17557 and Dublin soil 7084, and was similar to that found with Yolo soil 431 (compare tables 5 and 9). With the most concentrated solution, however, the Dublin soil absorbed by far the greatest amount of Na. As was to be expected, the soil having the lowest absorptive power (No. 6296) absorbed much the least Na. Soils 17557 and 7084 released to the solutions widely different amounts of Ca and Mg. In the case of soil 7084, the sum of all bases released ($\text{Ca} + \text{Mg} + \text{K}$) exceeded the Na absorbed by approximately 5 m.e. per liter. The results of this experiment afford a striking confirmation of the point discussed in the preceding paragraphs.

In the next experiment the ratio of Na to Ca of the solution was varied, the same general technic being used as in the preceding experiment. The results on Na absorption obtained with soil 17557 agree very well with those obtained with soil 431 (compare tables 7 and 10), but not as regards Mg release. On the other hand, with soils 7084 and 6296 the results were substantially different. Soil 7084 absorbed considerably the greatest absolute amount of Na from the more concentrated solutions and released much the greatest amount of Mg, both absolutely and relatively, whereas soil 6296 absorbed much the least amount of Na from all the solutions and at the same time released much less Mg than the other soils.

These results indicate that the initial effects of a given saline irrigation water may be quite different when applied to different types of soil. With soil low

in replaceable Mg and Ca (No. 6296), comparatively little Na was absorbed even from a solution containing 4 Na: 1 Ca. On the other hand, with soil high in replaceable Mg, much more Na is likely to be absorbed.

To test the permanency of the aforementioned effect, 20 gm. each of soils 431 and 7084 were leached with 2 liters of solutions containing NaCl and CaCl_2 in the ratio of 1:1 and 2:1. The ammonium acetate extractable bases of these soils were then determined. The effects produced by this treatment probably closely approach the limiting, or most extreme, effects that can possibly be produced on soils of these types by saline solutions of the designated composition and concentration. Table 11 shows that, after leaching with corresponding solutions, both the Yolo 431 and the Dublin 7084 soils were left with practically the same content of absorbed Na. Since soil 7084 originally, however, contained considerable absorbed Na, leaching with each of

TABLE 9
Effect of concentration of NaCl on different soils

SOIL NUMBER	CONCENTRATION APPLIED	SOLUTION OBTAINED					Na AB-SORBED
		Ca	Mg	K	Na	Total bases	
	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
17557	10.4	0.95	2.12	0.20	7.90	11.17	2.50
	20.8	1.60	3.50	0.30	16.10	21.50	4.70
	41.6	2.62	5.45	0.40	34.60	43.07	7.00
7084	10.4	2.55	4.54	0.18	7.80	15.07	2.60
	20.8	3.65	6.10	0.20	15.40	25.35	5.40
	41.6	5.65	9.40	0.19	31.60	46.84	10.00
6296	10.4	0.84	0.42	0.24	9.25	10.75	1.15
	20.8	1.29	0.56	0.32	18.40	20.57	2.40
	41.6	1.92	0.73	0.36	37.60	40.61	4.00

these solutions reduced the content of absorbed Na, whereas the 2:1 solution produced an opposite kind of effect on Yolo soil 431. In neither case was the final content of absorbed Na especially high. It was also found that leaching with a solution containing 2 Na to 1 Ca brought about a substantial increase in absorbed Ca, whereas the Mg content was reduced and the K was affected but little.

Insofar as base exchange is concerned, therefore, an irrigation water containing Na and Ca in a ratio of 2:1 will probably not permanently affect the physical properties of these soils adversely, provided a similar ratio and concentration of these ions prevail in the resulting soil moisture. Since the sum of Ca, K, and Na found in the leached Dublin soil (No. 7084) was approximately equal to the base-exchange capacity of this soil, the probability is that the excess of bases found, above the amount corresponding to the exchange

capacity, was primarily due to soluble Mg compounds. This excess was diminished by leaching with the saline solution, because of the removal of a part of the soluble Mg.

The original Dublin soil probably contained considerable replaceable Mg, since the sum of Ca, K, and Na found in it was less than the base-exchange

TABLE 10
Effect on different soils of solutions containing Na and Ca in various ratios

SOIL NUMBER	SOLUTION APPLIED		SOLUTION OBTAINED					Na ABSORBED
	NaCl	CaCl ₂	Ca	Mg	K	Na	Total bases	
17557	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
	10.4	10.4	5.84	5.65	0.34	8.80	20.63	1.60
	20.8	10.4	6.24	6.70	0.32	18.30	31.56	2.50
7084	41.6	10.4	7.32	7.62	0.40	36.50	51.84	5.10
	10.4	10.4	6.50	9.65	0.22	8.80	25.17	1.60
	20.8	10.4	7.90	10.10	0.21	16.50	34.71	4.30
6296	41.6	10.4	9.65	13.20	0.21	32.60	55.66	9.00
	10.4	10.4	9.60	1.15	0.25	10.10	21.10	0.30
	20.8	10.4	9.88	1.18	0.33	20.40	31.79	0.40
	41.6	10.4	10.40	1.23	0.40	40.90	52.93	0.70

TABLE 11
Effect on bases extractable with ammonium acetate produced by leaching soils with Na-Ca solutions

SOIL NUMBER	LEACHING SOLUTIONS		BASES EXTRACTED WITH AMMONIUM ACETATE					NH ₄ ABSORBED
	NaCl	CaCl ₂	Ca	Mg	K	Na	Total	
431	<i>m.e.</i>	<i>m.e.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e.</i>
	None*	None	21.70	6.00	1.36	1.22	30.28	28.30
	10.4	10.4	24.80	1.11	1.14	0.92	27.97	28.30
7084	20.80	10.4	25.00	0.65	1.06	1.63	28.34	28.10
	None*	None	38.98	37.75	0.94	3.19	80.86	54.80
	10.4	10.4	53.00	16.90	0.72	1.00	71.62	55.00
	20.8	10.4	53.50	10.20	0.62	1.85	66.17	55.20

* Data in these rows are for soils before leaching.

capacity (NH₄ absorbed). This accounts, in part, for the marked increase in exchangeable Ca following the treatment with the saline solutions.

In the next experiment chloride solutions containing variable ratios of Na to (Ca + Mg) were shaken to equilibrium with the soil, then analyzed. The results show that the partial substitution of Ca by Mg in the solutions had

but little effect on the absorption of Na (compare tables 7 and 12). This substitution, however, did have a marked effect on the replaceable Ca of the soil, as is shown by the fact that the Ca found in the extracts was much greater than in the original solutions. In this experiment the absorption of Na took place chiefly at the expense of the Ca of the soil, whereas with Na-Ca solutions (table 7) the absorption of Na was largely a consequence of Mg replacement.

TABLE 12

Effect of solutions containing various ratios of Na:(Ca + Mg) on soil 431

SOLUTION USED			SOLUTION OBTAINED					Na ABSORBED
Na	Ca	Mg	Ca	Mg	K	Na	Total	
<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>
10.4	5.2	5.2	9.15	5.74	0.85	9.20	24.94	1.20
20.8	5.2	5.2	10.05	6.30	0.86	18.50	35.71	2.30
41.6	5.2	5.2	10.90	6.20	0.88	37.00	54.98	4.60
10.4	7.8	2.6	10.28	4.80	0.78	11.10	26.96	-0.70
20.8	7.8	2.6	10.64	4.92	0.81	20.30	36.67	0.50
41.6	7.8	2.6	12.96	4.83	0.82	37.60	56.21	4.00

TABLE 13

Effect on soil 431 produced by leaching with Na-Ca-Mg solutions

LEACHING SOLUTIONS			BASES EXTRACTED WITH AMMONIUM ACETATE					NH ₄ ABSORBED
NaCl	CaCl ₂	MgCl ₂	Ca	Mg	K	Na	Total	
<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e./100 gm.</i>	<i>m.e.</i>
None*	None	None	21.70	6.00	1.36	1.22	30.28	28.30
10.4	5.2	5.2	15.20	8.25	1.06	2.25	26.76	28.75
20.8	5.2	5.2	15.25	8.41	1.03	2.42	27.11	28.85
41.6	5.2	5.2	15.75	8.25	1.10	3.14	28.24	28.75
10.4	7.8	2.6	18.65	5.70	1.10	2.34	27.79	28.15
20.8	7.8	2.6	19.00	5.35	1.05	2.62	28.02	28.23
41.6	7.8	2.6	19.25	5.00	1.12	3.13	28.50	28.23

* Data in this row are for soil before leaching.

Results of a study of the effects produced by leaching the soil with solutions containing the same ratios of Na to (Ca + Mg) as were used in the preceding experiment are reported in table 13. When these data are compared with those for the same soil recorded in table 11, it is seen that leaching with Na-Ca-Mg solutions caused the absorption of somewhat greater amounts of Na than did leaching with Na-Ca solutions of corresponding Na concentration. It is also shown that these two types of solution produced markedly different effects on the replaceable Ca and Mg of the soil. As has been pointed out,

leaching with Na-Ca solutions produced substantial increases in the replaceable Ca of the soil and reduced the replaceable Mg to a low level, whereas, as was to be expected, leaching with Na-Ca-Mg solutions reduced the content of replaceable Ca. Those solutions which contained Ca and Mg in the ratio of 1:1 in addition to Na produced an increase in the replaceable Mg content, whereas those which contained Ca and Mg in the ratio of 3:1 reduced the content of Mg somewhat. The reduced content of replaceable Ca and the increased content of replaceable Na produced by these solutions indicate that irrigation waters containing Na, Ca, and Mg might produce deleterious effects on this soil even though the ratio of Na to (Ca + Mg) is not greater than 1:1.

Consideration should be given, therefore, not only to the ratio of Na to the total alkali earth bases in irrigation waters, but also to the ratio of Ca to Mg. It is true the available evidence is that a relatively high content of replaceable Mg in soils is far less objectionable than a high content of Na; nevertheless, the replacement of Ca by Mg cannot be completely disregarded, and where this takes place concurrently with a substantial increase in replaceable Na, the net effect is likely to be decidedly unfavorable agronomically.

DISCUSSION

Reference has already been made to the fact that under field conditions the total concentration of the so-called soil solution is likely to exceed that of irrigation water applied. The degree to which this will be realized varies widely, depending on soil type and climatic conditions. Investigations to be reported later indicate that with relatively permeable soil, under California climatic conditions, the concentration of the salts in the soil moisture a few days after irrigation water has been applied is likely to become from three to six times that of the water applied. The foregoing results suggest that this increase in the concentration may be important for the reason that, as the concentration increases, the tendency is for the soil to acquire increased amounts of absorbed Na. Moreover, since the concentration of the soil moisture continues to increase as evaporation and transpiration proceed, the concentration of the thin films of moisture remaining in the soil when it approaches the wilting point may be ten or more times that of the water applied. It is to be borne in mind, however, that upon applying irrigation water anew the tendency will be for the divalent cations of the water applied to replace some of the Na that has been previously absorbed from the more concentrated soil solutions, especially in the surface horizon.

An additional factor must also be considered, namely, the kind of anions in the irrigation water; for example, if the soluble anion of the soil, whether native thereto or resulting from irrigation water, is entirely Cl^- , the concentrating effect resulting from evaporation and transpiration probably will not alter the ratio of Na to Ca in the liquid phase of the soil. On the other hand, if the irrigation water contains a substantial amount of HCO_3^- , which is commonly the case, or HCO_3^- is formed in the soil biologically, evaporation

may produce substantial alteration in the ratio of Na to Ca in the solution phase of the soil, because of the precipitation of CaCO_3 . Sulfate waters tend to act in a similar way because of the relatively low solubility of CaSO_4 . Therefore, the ratio of Na to Ca in the films of moisture which undergo displacement downward, either when irrigation water is applied or rains fall, is likely to be considerably wider than that of the water applied.

In view of these facts, it is probable that the ratio of Na to $(\text{Ca} + \text{Mg})$ in an irrigation supply should not exceed approximately 1:1. It is interesting to note that Scofield and Headley (5) and Eaton (1) have drawn a similar conclusion on the basis of field observations.

It remains to be noted that the nature of the anions of irrigation water is important for reasons additional to those already mentioned. The reference here is to the direct effect of anions on crop growth. It is well established that certain anions, BO_3^- for example, are extremely toxic to crops. CO_3^- is also comparatively toxic, partly because of its effect on the pH of the soil. The concentration of Cl^- and of SO_4^- may also become so high as to be distinctly toxic. Since the toxic effects of all these anions is much enhanced by increasing their concentration, and since the normal processes of soils result in the production of a soil solution concentration considerably greater than that of the irrigation water applied, the total concentration and specific kinds of anions in irrigation supplies are matters of primary importance.

SUMMARY

The dissolved salines of irrigation water are agriculturally important for two reasons, namely, because of their effect on the concentration and composition of the soil solution, and their effect on the absorbed bases of the soil. With a given irrigation water, the former is greatly influenced, and the latter to some extent, by the inherent properties of the soil, its permeability and profile characteristics, the climatic conditions, and the amount of the water applied. Since soils differ widely in regard to these points, and since different crops differ in their sensitivity to salines, no hard and fast line can be laid down as to permissible salinity of irrigation water.

Sodium salt solutions react with soils by base exchange with resulting absorption of Na by the soil. This effect increases with increasing concentration of the solution and also as the ratio of Na:Ca increases. The presence of absorbed Na in soils tends to affect their physical properties adversely.

The ratio of Na to Ca in the solution has relatively great influence on the absorption of Na. If this ratio is not greater than 2 to 1, very little Na will be absorbed, but as this ratio exceeds 2 to 1 the absorption of Na tends to increase proportionately.

The kind of base held by the soil in replaceable form influences the absorption of Na. If the soil is Ca saturated, less Na will be absorbed from a given solution than if it is Mg saturated. If Mg constitutes a relatively high percentage of the total replaceable bases of the soil, relatively much Na will be

absorbed. The Na of a solution of a given concentration, in which Ca is the only divalent base present, will be absorbed by a soil to less extent than if a substantial part of the divalent base is Mg. These facts follow from the differential replacing power of these bases.

Other conditions being equal, less Na will be absorbed by calcareous than by noncalcareous soils, because CaCO_3 tends to yield to the solution Ca ions, which in turn repress the absorption of Na.

Despite the fact that very little Na is absorbed from saline solutions in which the ratio of Na:Ca is not greater than 2:1, it does not follow that the application of a comparatively dilute saline irrigation water containing Na and Ca + Mg in the ratio of 2:1 will have no deleterious effect on the soil. The reason why this is true is twofold: first, the concentration of the salts in the soil moisture is sure to exceed that of the water applied; second, the ratio of Na to divalent bases in the resulting soil moisture is likely, because of the precipitation of Ca salts and the absorption of Ca by crops, to become considerably greater than that of the water applied. For these reasons it is suggested that the ratio of Na to Ca + Mg should not exceed about 1:1, but this conclusion should not be interpreted too rigorously because of the very great influence of total concentration and of different anions. With very dilute irrigation water it is probable that this ratio may safely be somewhat greater than 1:1.

REFERENCES

- (1) EATON, F. M. 1935 Boron in soils and irrigation waters and its effect on plants, with particular reference to the San Joaquin Valley of California. U. S. Dept. Agr. Tech. Bul. 448.
- (2) FRAPS, G. S., AND FUDGE, J. F. 1938 Replacement of calcium in soils by sodium from synthetic irrigation water. *Jour. Amer. Soc. Agron.* 30: 789-796.
- (3) KELLEY, W. P., AND CUMMINS, A. B. 1921 Chemical effect of salts on soils. *Soil Sci.* 11: 139-159.
- (4) PUFFELES, M. 1939 Effect of saline water on Mediterranean loess soils. *Soil Sci.* 47: 447-453.
- (5) SCOTFIELD, C. S., AND HEADLEY, F. B. 1921 Quality of irrigation water in relation to land reclamation. *Jour. Agr. Res.* 21: 265-278.

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XXI A. THE AMPHOTERIC POINTS, THE pH, AND THE DONNAN EQUILIBRIUM

Part A. Theoretical

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In previous papers (4, 5) a theoretical analysis of the interrelationships of the amphoteric points of soils was given for the case (a) that the cations of the added salt solution are more strongly adsorbed than the anions, and vice versa, and (b) that the acidoid equivalence of the soil is greater than the basoid equivalence, and vice versa. In this paper we present the results of a study of the relationship of the mass law, as expressed in the form of the Donnan equilibrium, to the equi-ionic point and the point of exchange neutrality and to the effect of salts on the pH of soils.

We shall begin with a definition of the amphoteric points, followed by an experimental illustration.

THE AMPHOTERIC POINTS

The equi-ionic point is defined as that pH of a salt solution which is not affected by the addition of the completely unsaturated soil (free acid-base ampholytoid). It represents the pH at which the capacities of the soil to combine with the anions and the cations of the solution are equal.

The pH of exchange neutrality is that point at which the addition of a neutral salt to a soil suspension does not affect the pH of the latter. It represents the pH at which the increments, produced by the salt, in the capacities of the soil to combine with the anions and the cations of the solution are equal.

The relationship in the case of a certain soil is brought out in figure 76. The curves were obtained by titrating the electrodialyzed material from the Haggbygget podzol B₂ horizon by NaOH and H₂SO₄ in water and in the presence of *N* Na₂SO₄. In addition to the soil curves, the curve obtained by titrating the strong acid by the strong base (the solution curve) has also been plotted.

We note that each of the soil curves intersects the solution curve. *The point of intersection represents that pH of the solution which is unaffected by the addition of the soil.* Below this point the soil causes an increase in the pH of the solution; above, it causes a decrease in the pH. Since the soil was in the electrodialyzed, free acidoid-basoid condition, it follows that it binds acid

as well as base and that at the point of intersection with the solution curve the soil binds an equivalent quantity of acid and base (anions and cations).

This point, which was originally defined as the pH of exchange neutrality, we now define as *the equi-ionic point*, thereby signifying that the soil at this pH binds equivalent quantities of anions and cations.

The point of intersection of the soil curves, E_x , we define as *the point of exchange neutrality*, since it represents that pH of a soil suspension which is unaffected by the addition of the salt. At this point the soil is exchange-neutral in its reaction toward the salt.

Before we take up the complicated problem of the relationship of the mass law to the amphoteric points of soils, we shall find it helpful to make a more

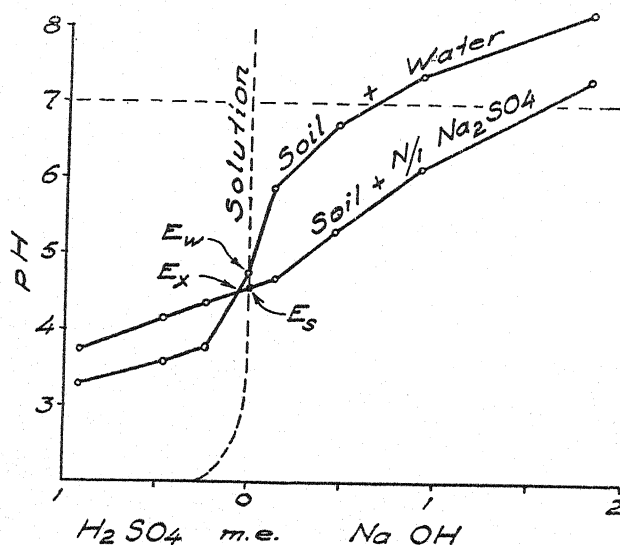


FIG. 76. TITRATION OF THE HÄGGBYGGET PODZOL (B_2) BY NaOH AND H_2SO_4 IN WATER AND IN $N \text{ Na}_2\text{SO}_4$

10 gm. soil in 20 cc. (E_w and E_s = equi-ionic points, E_x = point of exchange neutrality)

detailed study of these points themselves by placing the problem on an experimental basis analogous to the theoretical treatment.

The titration curves in figure 76 show only the *net* capacity of the soil to bind base and acid ($x - y$). The individual or *absolute* capacities of the acidoid groups to bind base (x) and of the basoid groups to bind acid (y) are not shown. In the following experiment, both of these series of values have been determined by shaking the soil (Häggbygget B_2 sample) in approximately 0.002 and 0.2 N $(\text{NH}_4)_2\text{SO}_4$ in two series, in which the pH was varied by the addition of NH_4OH and H_2SO_4 .

The dilute series contained 10 gm. soil and 1.962 m.e. $(\text{NH}_4)_2\text{SO}_4$ in a total volume of 1000 cc. solution, and the concentrated series contained 100 gm.

soil and 19.742 m.e. $(\text{NH}_4)_2\text{SO}_4$ in a total volume of 100 cc. solution. The proportion of soil to salt was, therefore, about the same in both series, the only difference being that the dilution was 100 times greater in the diluted series. The suspensions were shaken 18 hours the first day and 2 hours the second and third days and then were filtered through a 00 Berzelius filter paper under conditions which prevented evaporation. Though the concentrated suspensions would not yield a clear filtrate above a pH of 6, we succeeded fairly well in covering the amphoteric range of the soil. The pH was determined by the quinhydrone method, the NH_3 by distillation, and the SO_4 as BaSO_4 . The results are shown in table 152.

If we plot, against the pH, the adsorbed quantities x and x' of NH_4OH in the dilute and concentrated series respectively and the adsorbed quantities y and y' of H_2SO_4 in the corresponding series we get the four unbroken curves in figure 77. These curves show the *absolute* amounts of acid and base adsorbed by the soil at different pH values. If we then plot the $x - y$ and $x' - y'$ values given in the last column of the table we obtain the broken curves in the figure. These curves correspond to the titration curves in figure 76 and express the *net* amount of adsorbed acid (negative values) and base (positive values). The titration curves in figure 76 differ, however, from the $x - y$ curves in figure 77 in that the former express the free acid and base in addition to the adsorbed, that is, they express the buffer effect of the whole system. The titration curve may be said to represent the sum of the $x - y$ curve and the solution curve in figure 76.

The equi-ionic points, $x - y = 0$ (dilute series) and $x' - y' = 0$ (concentrated series), are designated by E_s and $E_{s'}$ respectively. The equi-ionic point in the dilute solution is at pH 4.60, and in the concentrated solution at about 4.20. This downward deflection of the equi-ionic point is due to a greater equivalence of acidoid than of basoid groups in the soil.

It must, however, be pointed out that, at low pH, the combination between the basoid group and the H_2SO_4 is greater than is indicated by the adsorption. This is due to the cationic solvation of the ionized basoid groups which carries the adsorbed SO_4 ion back into solution. In the dilute series this solvation attained such proportions at a pH below 3.5 as to show a decrease in the amount of SO_4 ions adsorbed. This does not manifest itself in the concentrated series, within the range covered, because the high concentration of the divalent SO_4 ions keeps the cationic complex in the gel condition, just as Ca ions would keep the anionic complex in the coagulated condition at a pH above the equi-ionic point. A similar apparent decrease in the adsorption would be observed at a high pH in the case of the NH_4 ion, which here is carried back into solution through the anionic solvation of the ionized acidoid groups.

It is interesting to compare the saturation of the soil at the equi-ionic points of the two series. At the equi-ionic point in the dilute solution (E_s) the saturation is 0.4 m.e. acid and base per 100 gm. soil, whereas it is about 4.20 m.e., or more than ten times as great, at the equi-ionic point in the

concentrated solution (E_s). The latter value is over 50 per cent of the capacity of the soil to bind NH_4 at pH 7.0 in the dilute solution, which amounts to about 7.75 m.e. per 100 gm. The results show how greatly the adsorption of anions and cations can overlap.

Figure 77 makes the relationship between the equi-ionic points (E_s and $E_{s'}$) and the point of exchange neutrality (E_x) very clear. Suppose that the soil is at the equi-ionic point in the dilute solution (at E_s). The pH is 4.6, and the soil has adsorbed 0.4 m.e. acid and base ($x = y = 0.4$). Suppose then that the concentration is made equal to that of the concentrated solution by the addition of salt. What will happen? At pH 4.6, in the concentrated

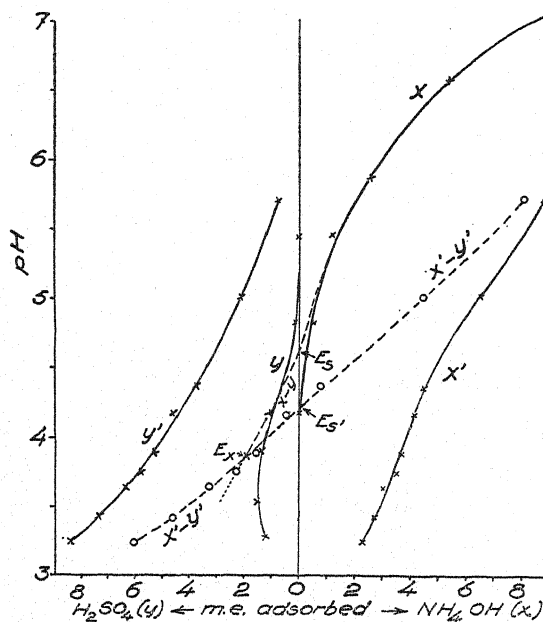


FIG. 77. ADSORPTION OF NH_4 AND SO_4 IONS FROM 0.002 N (x AND y) AND 0.2 N (x' AND y') SOLUTIONS OF $(\text{NH}_4)_2\text{SO}_4$ BY THE B₂ HÄGGBYGGET PODZOL
(Cf. table 152 and fig. 76)

solution, the soil binds $x' = 5.5$ m.e. base and $y' = 3.1$ m.e. acid or 2.4 m.e. more base than acid (or cations than anions). The obvious result is an exchange acidity. But the capacity to bind acid increases with a lowering of the pH, whereas the capacity to bind base decreases. If, therefore, we lower the pH of the dilute system by the addition of acid we shall eventually reach a point at which the addition of salt causes the same *increment* in the anion and cation adsorption and at which the soil, therefore, is exchange-neutral in reaction. This will happen at the pH where

$$(x' - x) - (y' - y) = 0$$

and is represented in figure 77 by the intersection of the $x - y$ and $x' - y'$ curves. At the point E_x (pH about 3.85) $x' = 3.7$, $x = 0$, $y' = 5.2$, and $y = 1.5$ (approximate, interpolated values) and

$$(3.7 - 0) - (5.2 - 1.5) = 0.$$

A little reflection will show that whenever the cations are more extensively adsorbed than the anions, that is, when the equi-ionic point in the more

TABLE 152

Adsorption of NH_4 and SO_4 ions from dilute and concentrated solutions of $(\text{NH}_4)_2\text{SO}_4$ by the B_2 Hügbygget podzol

A. Dilute series (10 gm. soil, 1962 m.e. $(\text{NH}_4)_2\text{SO}_4$, 1000 cc. solution)

NUMBER	pH	ADDED M.E./10 GM.		ADSORBED M.E./100 GM.		$x - y$
		NH_4OH	H_2SO_4	NH_4OH (x)	H_2SO_4 (y)	
1	3.27	3.00	1.21	-1.21
2	3.53	2.00	1.47	-1.47
3	3.89	1.00	1.36	-1.36
4	4.17	0.50	1.03	-1.03
5	4.81	0.52	0.10	0.42
6	5.43	0.213	1.21	1.21
7	5.84	0.426	2.57	2.57
8	6.55	0.852	5.41	5.41
9	7.67	1.704	11.10	11.10

B. Concentrated series (100 gm. soil, 19.742 m.e. $(\text{NH}_4)_2\text{SO}_4$, 100 cc. solution)

NUMBER	pH	ADDED M.E./100 GM.		ADSORBED M.E./100 GM.		$x' - y'$
		NH_4OH	H_2SO_4	NH_4OH (x')	H_2SO_4 (y')	
1	3.24	20.20	2.351	8.382	-6.031
2	3.41	14.14	2.732	7.332	-4.600
3	3.63	8.00	3.100	6.372	-3.272
4	3.73	6.00	3.562	5.782	-2.220
5	3.87	4.00	3.723	5.242	-1.519
6	4.15	1.00	4.156	4.612	-0.456
7	4.35	4.498	3.712	0.786
8	4.99	4.258	6.610	2.102	4.508
9	5.69	8.515	8.820	0.742	8.078

concentrated solution lies below the equi-ionic point in the dilute solution, then the point of exchange neutrality will be on the acid side of the equi-ionic points. Suppose that the pH of the dilute system were adjusted to the equi-ionic point of the concentrated system, i.e., to pH 4.2. The soil would bind 1 m.e. acid but no base ($y = 1$, $x = 0$). Now let the concentration be increased to the higher value by the addition of the salt. At pH 4.2 the

soil would now bind 4.20 m.e. acid and 4.20 m.e. base ($y' = 4.20$, $x' = 4.20$). But having already adsorbed 1 m.e. of the previously added acid it would only adsorb an increment of 3.20 m.e. of the anions to 4.20 m.e. of the cations of the salt. The salt would, even here, produce an exchange acidity. It is not until we get down to a pH of 3.85 (E_s) that the soil will adsorb (in exchange for its OH and H ions) an equal number of the anions and cations of the added salt.

The same reasoning applied to the case where the anions of the added salt are more extensively adsorbed than the cations will show that the point of exchange neutrality will then lie above the equi-ionic points.

THE DONNAN EQUILIBRIUM

In our previous papers we have assumed that the saloids are incompletely dissociated, that the Ca-saloid is less dissociated than the Na-saloid and so forth. The charge, the power to imbibe water, and other colloidal properties point that way. But it is also possible that the saloids, like most of the salts, are completely dissociated. It seems probable that monovalent groups on the surface of the acidoid are so far apart that a complete association with a divalent cation is impossible. Thus the phosphate-bound Ca in a phosphoric acidoid (Fe- and Al-phosphates) is easily displaced by the alkali cations (6).

Yet, because of their very nature, the saloids may, in effect, be incompletely dissociated, inasmuch as the micellar ions are within the range of attraction of the colloidal ion-complex. The interionic attraction must markedly reduce the activity coefficient (f) of the ions, and this means a low activity (fc) in proportion to the concentration (c). The divalent ions, which may be assumed to be held nearer the surface than the monovalent ions, would therefore suffer the greatest reduction in their activity. The lower the activity of an ion in the micellar solution, the greater will be the displacing power of that ion when added to the system.

Such a partial dissociation of all the ions would equally well serve to account for the variations in the apparent dissociation constants of the acidoid and basoid groups and for the position of the amphoteric points of soils in different salt solutions. An application of the mass law would lead to the same results as in the case of our previous assumption of a limited dissociation, provided that we substitute the activity in place of concentration.

But even on the basis of a complete dissociation of the saloids, we can, by an application of the mass law in the form of the Donnan equilibrium to the distribution of the free ions of the system—an application which has led to some very significant and interesting results—, account in a general way for the phenomena here studied.

The application of the Donnan equilibrium to the study of cation exchange in soils has been made by Teräsvuori (9) and Möller (7). Valuable and interesting as this work is, it can hardly be considered to have passed the qualitative stage. Du Rietz (1), who used a lignosulfonic acidoid (a strong,

completely dissociated acidoid), has been able to place the problem on a quantitative basis and thus contribute greatly to its solution. The results obtained by these workers leave no doubt that the classical mass law in its modern formulation can be applied even in the case of so complex a material as the soil. In the following discussion, therefore, we shall apply the Donnan equilibrium to the amphoteric reactions of soils in order to show what consequences may be drawn and what conclusions may be reached.

We are aware of the fact that an application of the Donnan equilibrium to amphoteric colloids is extremely complicated, and we admit that it might seem difficult to understand how an amphoteric soil, which at the equi-ionic point binds large quantities of anions and cations, can possess an electrostatically attracted swarm of free ions of opposite sign of charge. Why do not these ions, pairwise, distribute themselves equally throughout the system, since the colloidal ions would compensate each other like amphoteric "Zwitterionen"? But the acidoid and basoid ions may be too far apart to exert any appreciable interionic attraction. In this case these ions will attract the diffusible ions of opposite sign of charge and thus form an "amphoteric" ion atmosphere within which the anions and cations alternately dominate from point to point. The soil particle may also consist of a mosaic of acidoid and basoid clusters outside which "clouds" of cations (over the acidoid areas) and of anions (over the basoid areas) would gather. This would be the case especially in the more heterogenous, less intimate mixtures of acidoids and basoids.

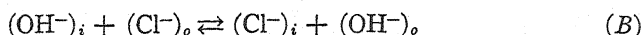
In the following discussion we shall assume that the amphoteric soil particle possesses, at and near the equi-ionic point, swarms of dissociated anions and cations.

The ions which swarm around the particles show no selectivity toward the colloidal ion as in the formation of a crystal lattice or a slightly dissociated compound. Any other ion of the same sign of charge will compensate the colloidal ion equally well and will, therefore, when it comes near the surface, allow the ion originally dissociated by the colloid to diffuse away. Within the micellar solution there is no difference between the ions "belonging" to a salt present in the system and the ions "belonging" to the colloidal ions. The micellar solution merely contains an excess of ions of opposite sign of charge to that of the colloidal ions. The concentration of the micellar solution (which contains the swarm ions together with the ions of the free electrolyte) is always greater than that of the outside solution, but the two concentrations become almost equal at high concentrations. The composition of the micellar solution will depend upon the composition of the outside solution because there is a continual exchange of ions according to the mass law. For monovalent ions this exchange may be formulated as follows:

for an acidoid



and for a basoid



where the parenthesis stands for the ion activity and i and o signify that the ion is in the inside and the outside solution respectively.

The Donnan equilibrium then gives:

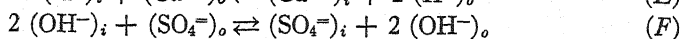
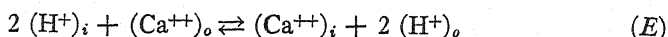
for the acidoid

$$\frac{(\text{H}^+)_i \cdot (\text{Na}^+)_o}{(\text{H}^+)_o \cdot (\text{Na}^+)_i} = K \quad (C)$$

and for the basoid

$$\frac{(\text{OH}^-)_i \cdot (\text{Cl}^-)_o}{(\text{OH}^-)_o \cdot (\text{Cl}^-)_i} = K \quad (D)$$

When the H and OH ions are displaced by divalent ions the corresponding equations become:



from which the corresponding Donnan expressions assume the following forms:

$$\frac{(\text{H}^+)_i^2 \cdot (\text{Ca}^{++})_o}{(\text{H}^+)_o^2 \cdot (\text{Ca}^{++})_i} = K \quad (G)$$

and

$$\frac{(\text{OH}^-)_i^2 \cdot (\text{SO}_4^{--})_o}{(\text{OH}^-)_o^2 \cdot (\text{SO}_4^{--})_i} = K \quad (H)$$

The Donnan distribution of the ions between the micellar and the outside solution may also be expressed as follows:
for the acidoid

$$\frac{(\text{H}^+)_i}{(\text{H}^+)_o} = \frac{(\text{Na}^+)_i}{(\text{Na}^+)_o} = \frac{\sqrt{(\text{Ca}^{++})_i}}{\sqrt{(\text{Ca}^{++})_o}} \quad (I)$$

and for the basoid

$$\frac{(\text{OH}^-)_i}{(\text{OH}^-)_o} = \frac{(\text{Cl}^-)_i}{(\text{Cl}^-)_o} = \frac{\sqrt{(\text{SO}_4^{--})_i}}{\sqrt{(\text{SO}_4^{--})_o}} \quad (J)$$

In studying the distribution of the ions of the free electrolyte between bentonite gel and the outside solution the following equations were used by Mattson (2, 3):

$$x^2 = y(y + z) \quad (K)$$

when all the ions are monovalent, e.g., Na-saturated bentonite in a NaCl solution; and

$$x^3 = y^2(y + z) \quad (L)$$

when the cation is divalent, e.g., Ca-saturated bentonite in a CaCl_2 solution.

Here x is the activity of the anions and cations of the free salt in the outside solution, y their activity in the micellar solution, and z the activity in the micellar solution of the cation dissociated by the acidoid.

In terms of x , y , and z , equation (I) becomes:¹

$$\frac{y_{\text{Na}} + z_{\text{Na}}}{x_{\text{Na}}} = \frac{\sqrt{y_{\text{Ca}} + z_{\text{Ca}}}}{\sqrt{x_{\text{Ca}}}} \quad (M)$$

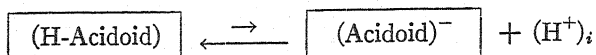
In equations (I) and (J) the divalent ions enter as the square root of their activity. This means that a dilution, which affects the outside solution much more than the inside, will favor the entrance of the divalent and the outgo of the monovalent ions. That is, the relative displacing power of divalent ions will be greatly enhanced in dilute solutions, whereas in high concentrations the displacing power of the two ions should be more nearly balanced, provided the different saloids are completely or equally dissociated. It is the application of this law to the amphoteric behavior of soils which is the object of our present investigation.

It should perhaps be pointed out that the far greater displacing power of the H (and OH) ions in no way invalidates the application of this law. Whatever our views on the soil saloids might be, it seems certain that the soil acidoids (and basoids) are dissociated to a limited extent. A low dissociation means a low activity in the micellar solution; and the lower this activity, the greater will be the displacing power of the H ions, because a correspondingly low activity in the outside solution will satisfy the relationship in equation (I). The free H ions are distributed according to the Donnan equilibrium similarly

¹ Because of a confusion of terms Mattson (2) disregarded the valence when calculating the Donnan potential and erroneously concluded that the results in table 10 of this series were in agreement with the theory. In this experiment the ratios of x/y were about the same for the Cl and the SO_4 ions (which were both present) although x was in excess of y by a little over 0.011 in a total concentration of about one-half normal. This is, as Schofield (8) points out, "a serious discrepancy . . . in the one case where an internal check can be made."

The intention is here merely to correct an erroneous conclusion, but it should also be pointed out that it is undoubtedly wrong to use so high concentrations in a study of the Donnan equilibrium. It is therefore not so surprising that the x/y ratios were the same for the two ions as that there should be any appreciable difference in the values of x and y . Or, is the "negative adsorption" due to two different causes: (a) to the fact that the "unfree" water does not act as solvent (in which case the valence would have no influence) and (b) to the Donnan distribution? Wiegner observed no negative adsorption when working on the basis of air-dry material. We hope to repeat the experiment in table 10 in dilute solutions and once more try to gain some definite information concerning this very important problem.

to the other monovalent ions, but their great displacing power is governed by the equilibrium:



The same applies to the OH ions of the soil basoids, which are even weaker than the soil acidoids.

From the fact (a) that a soil simultaneously adsorbs large quantities of anions and cations at the equi-ionic point in the presence of a neutral salt, and from the fact (b) that in dilute solutions the divalent anions and cations displace the OH and H ions of the soil much more strongly than do the monovalent anions and cations, whereas in stronger solutions the displacing power of the ions is more nearly balanced, we are led to very significant conclusions.

If to an unsaturated soil, whose acidoid and basoid groups are present in equivalent proportion, we add increasing concentrations of a neutral salt solution containing a divalent cation and a monovalent anion, e.g., CaCl_2 , we ought to get the following results:

In low concentrations the number of H ions displaced by the Ca ions will be considerably greater than the number of OH ions displaced by the Cl ions. In higher concentrations the two reactions will more nearly balance each other. The exchange acidity must therefore attain a maximum in dilute solutions and then decrease in higher concentrations.²

If we add a solution containing a divalent anion and a monovalent cation, e.g., Na_2SO_4 , we ought to get a maximum exchange alkalinity in dilute solution.

To test this theory we selected a sample from the B horizon of an iron podzol (from Furudal, Dalarna), which had the same pH in water and in 0.01 *N* NaCl solution. We took this as an indication of an equivalence between the acidoid and basoid groups. The pH was determined by the glass electrode in water and in various concentrations of Na_2SO_4 up to 2.0 *N*. The results, given in figure 78, show that a concentration of 0.02 *N* gave a maximum exchange alkalinity equal to 0.52 pH unit whereas a 100 times stronger solution gave a pH only 0.24 unit higher than that in water.

Determinations of the pH of various soils in water and in 0.01 *N* and *N* Na_2SO_4 gave the highest values in the *N* solution when the basoid group dominated (lateritic subsoils) and in the 0.01 *N* solution when the equivalence

² A disturbing factor, especially when the acidoid content is high and the exchange acidity great, will be the dissolution of the basoid-bound Cl ions in the form of AlCl_3 , which by hydrolysis gives HCl. Since this solution of Al (and Fe) is greater at a given pH the higher the concentration of salt, it is clear that the exchange acidity, which thus comes from two sources, will be enhanced in the concentrated solution. To the extent that this dissolution takes place, the exchange alkalinity resulting from a displacement of OH ions will be neutralized, and this will partly or completely obscure the valence effect as above postulated according to the Donnan equilibrium. This applies to all the neutral salts, but the effect will be smaller for salts of divalent anions and monovalent cations because of a higher pH and the coagulating effect of the divalent anions on the cationic sol complex.

between acidoids and basoids seemed to be balanced (lateritic surface soil and iron podzol samples from the B horizon). An excess of acidoids over basoids always resulted in an exchange acidity in both salt solutions (humus soils and gray soils). A little reflection will show that this is all in agreement with the theory. But the relationships are very complicated and will be more easily comprehended if we construct some theoretical curves analogous to the experimental curves in figure 77.

Knowing neither the activities nor the concentrations of the ions in the micellar solution and knowing of no reliable method by which they may be determined in so complex systems as soils, we shall not attempt a quantitative application of the foregoing equations in order to find how the experimental results fit the theory. But we can make a qualitative application of the mass law to an amphoteric soil in at least two different ways. We can construct

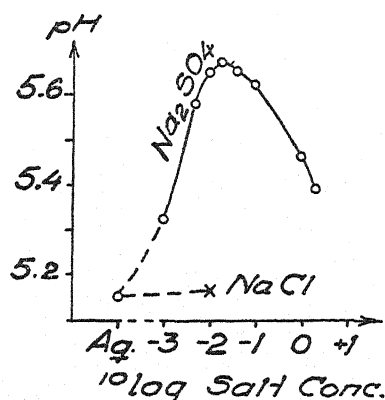


FIG. 78. THE pH OF THE FURUDAL SOIL IN WATER AND IN SOLUTIONS OF VARIOUS CONCENTRATIONS OF Na_2SO_4

titration (dissociation) curves which are based on apparent acid and base dissociation constants assumed in accordance with the valence effect in the Donnan equilibrium and we can calculate this equilibrium on the basis of various assumed activities in the inside and outside solutions.

The more strongly an ion displaces the H ions of a soil acidoid, the stronger will be the apparent dissociation constant of the acidoid and the greater will be the capacity of the soil to bind base at a given pH. The same applies to the basoids. Now it follows on the basis of the Donnan equation that, in low concentrations, divalent anions and cations must cause a much greater increase in the apparent basoid and acidoid dissociation constants than do the monovalent ions, whereas in high concentrations the increase will be more nearly the same.

That this is actually the case is shown in figures 79 and 80.³ The soil here

³ Taken from unpublished data by Mattson and Ekman.

used is from the same profile as that described in figures 76 and 77. It will be seen that 0.01 N CaCl_2 causes a very much greater increase in the exchange acidity and in the capacity to bind base than does 0.01 N NaCl , whereas the

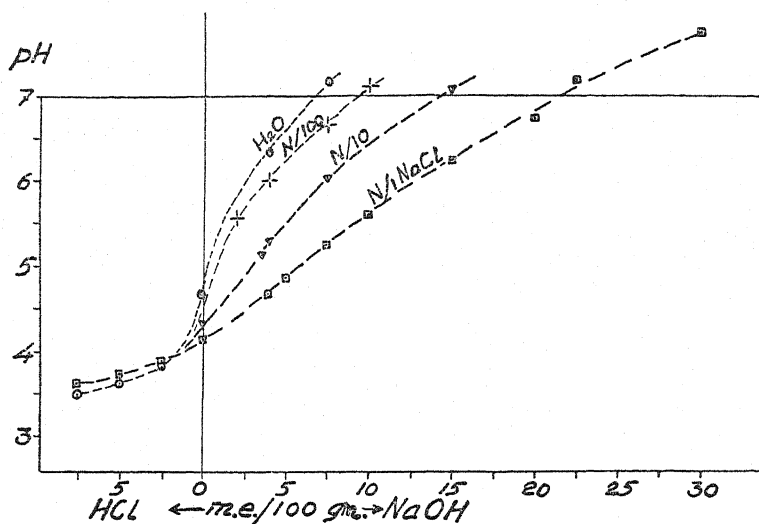


FIG. 79. TITRATION OF THE B₂ HÄGBYGGET PODZOL IN WATER AND IN 0.01, 0.1, AND 1 N NaCl

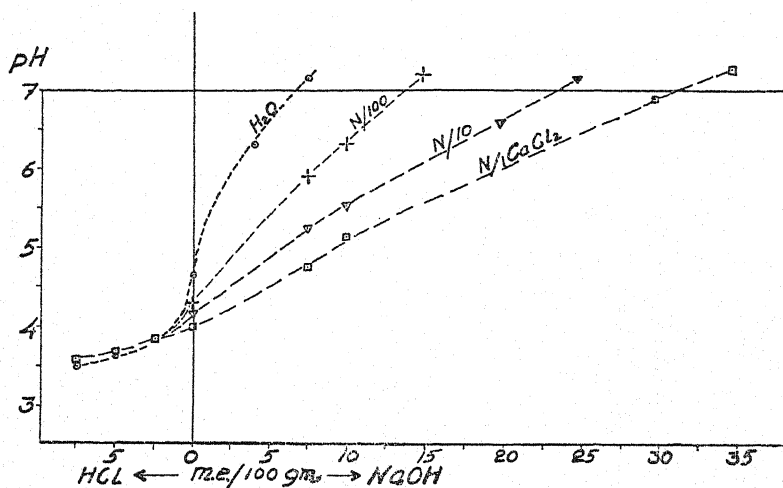


FIG. 80. TITRATION OF THE B₂ HÄGBYGGET PODZOL IN WATER AND IN 0.01, 0.1, AND 1 N CaCl_2

corresponding increments between 0.1 N and N are greatest in the case of NaCl . We present these curves merely to justify the following assumptions made as a basis for the theoretical curves in figure 83, and therefore we shall not at this time discuss any other details in figures 79 and 80.

The question now arises: How will this valence difference, in various combinations and concentrations of ions, affect the equi-ionic point and the point of exchange neutrality of the system?

To illustrate this we will first consider a system containing 1 mol of an acidoid H_3A and 1 mol of a basoid $B(OH)_3$ whose apparent dissociation constants k_a

TABLE 153
Theoretical "neutralization" of a system containing 1 mol of an acid H_3A
and 1 mol of a base $B(OH)_3$

Capacity to bind base (x) and acid (y) when:

$\frac{(H^+)}{(OH^-)}$	10^{-6} 10^{-14}	10^{-1} 10^{-13}	10^{-2} 10^{-12}	10^{-3} 10^{-11}	10^{-4} 10^{-10}	10^{-5} 10^{-9}	10^{-6} 10^{-8}	10^{-7} 10^{-7}	10^{-8} 10^{-6}	10^{-9} 10^{-5}	10^{-10} 10^{-4}
(A) $k_{a_1} = 1 \times 10^{-6}$, $k_{a_2} = 1 \times 10^{-7}$, $k_{a_3} = 1 \times 10^{-8}$, $c = 1M$											
x					.01	.10	.60	1.50	2.40	2.90	2.99
(B) $k_{b_1} = 1 \times 10^{-10}$, $k_{b_2} = 1 \times 10^{-11}$, $k_{b_3} = 1 \times 10^{-12}$, $c = 1M$											
y	2.99	2.90	2.40	1.50	.60	.10	.01				
$x-y$	-2.99	-2.90	-2.40	-1.50	-.59	± 0	.59	1.50	2.40	2.90	2.99
(A') $k'_{a_1} = 1.78 \times 10^{-6}$, $k'_{a_2} = 1.78 \times 10^{-7}$, $k'_{a_3} = 1.78 \times 10^{-8}$, $c = 1M$											
x'					.02	.17	.81	1.74	2.58	2.94	2.99
(B') $k'_{b_1} = 3.16 \times 10^{-9}$, $k'_{b_2} = 3.16 \times 10^{-10}$, $k'_{b_3} = 3.16 \times 10^{-11}$, $c = 1M$											
y'	3.00	3.00	2.97	2.73	1.97	1.03	.27	.03			
$x'-y'$	-3.00	-3.00	-2.97	-2.73	-1.95	-.86	.54	1.71	2.58	2.94	2.99
(A'') $k''_{a_1} = 1 \times 10^{-4}$, $k''_{a_2} = 1 \times 10^{-5}$, $k''_{a_3} = 1 \times 10^{-6}$, $c = 1M$											
x''			.01	.10	.60	1.50	2.40	2.90	2.99	3.00	3.00
(B'') $k''_{b_1} = 3.16 \times 10^{-8}$, $k''_{b_2} = 3.16 \times 10^{-9}$, $k''_{b_3} = 3.16 \times 10^{-10}$, $c = 1M$											
y''	3.00	3.00	3.00	2.97	2.73	1.97	1.03	.27	.03		
$x''-y''$	-3.00	-3.00	-2.99	-2.87	-2.13	-.47	1.37	2.63	2.96	3.00	3.00
$(x'-x)-(y'-y)$	-.01	-.10	-.57	-1.23	-1.36	-.86	-.05	.21	.18	.04	
$(x''-x)-(y''-y)$	-.01	-.10	-.59	-1.37	-1.54	-.47	.78	1.13	.56	.10	.01
$(x''-x')-(y''-y')$			-.02	-.14	-.18	.39	.83	.92	.38	.06	.01

and k_b and whose capacities to bind base (x)⁴ and acid (y)⁴ respectively, when titrated in water, are as given under (A) and (B) in table 153. If instead of

$$^4x = \frac{c}{1 + \frac{(H^+)}{k_a}}, \quad y = \frac{c}{1 + \frac{(OH^-)}{k_b}}$$

water we use a dilute solution of a salt of a divalent anion and a monovalent cation such as an alkali sulfate, the k_b values will increase considerably, whereas the k_a values will be much less affected [cf. (A') and (B') table 153]. In a strong solution of the same salt both the k_b and the k_a values will be greatly increased [cf. (A'') and (B'') table 153].

In terms of pk , the assumed values for the apparent dissociation constants in water and in solutions of a neutral salt of the type M^+S^{2-} are given in table 153 A.

By plotting the x and y values against the pH we get the curves in figure 83. The first notable thing here is the fact that the equi-ionic point ($x - y = 0$) in the dilute solution $E_{s'}$ is not only above the equi-ionic point in water E_w but is also above the equi-ionic point in the concentrated solution $E_{s''}$. (Cf. the various values for x and y and $x - y$ in the column under $(H^+) = 10^{-5}$ in table 153.) A soil having the corresponding amphoteric properties would, in the completely unsaturated condition, yield a greater exchange alkalinity upon the addition of a smaller than a larger amount of the salt, exactly as we have shown in figure 78.

TABLE 153 A

Assumed pk values in water and in solutions of a neutral salt of the type M^+S^{2-}

	pk_{a_1}	pk_{a_2}	pk_{a_3}	pk_{b_1}	pk_{b_2}	pk_{b_3}
In water.....	6.0	7.0	8.0	10.0	11.0	12.0
In dilute solution.....	5.75	6.75	7.75	8.5	9.5	10.5
In concentrated solution.....	4.0	5.0	6.0	7.5	8.5	9.5

From this we can make the further deduction that if the soil originally contained an amount of salt corresponding (when suspended in water) to that of the dilute solution and we then added salt to correspond to the concentrated solution we would find that the salt caused a lowering of the pH. If, however, we decided to leach the soil before determining the pH in water and in the solution we would find that the salt caused an elevation of the pH. In the first case we would find an exchange acidity and in the second an exchange alkalinity.

The differences in the *increments* produced by the salt in the capacity of the system to bind base (x) and acid (y) are given in the last three rows of table 153. Where these differences are positive the addition of the salt produces an exchange acidity; where negative, an exchange alkalinity. The point of exchange neutrality is the pH where this difference equals zero and corresponds to the point of intersection of each pair of curves in figure 83. In changing from water to the dilute solution and from water to the concentrated solution the points of exchange neutrality, $E_{x'}$ and $E_{x''}$ respectively, are both on the alkaline side of the corresponding equi-ionic points in our assumed system. In

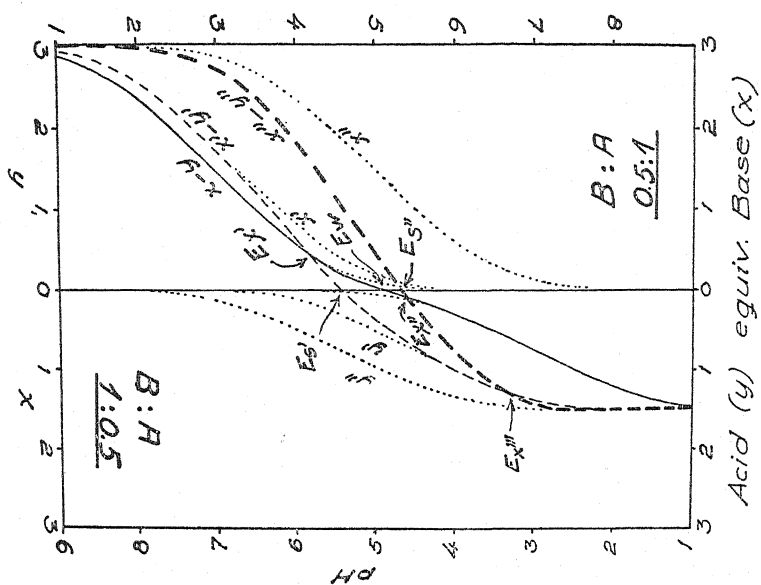


FIG. 81

FIG. 81. THE SAME AS FIGURE 83 WHEN BASOID: ACIDOID = 0.2:1

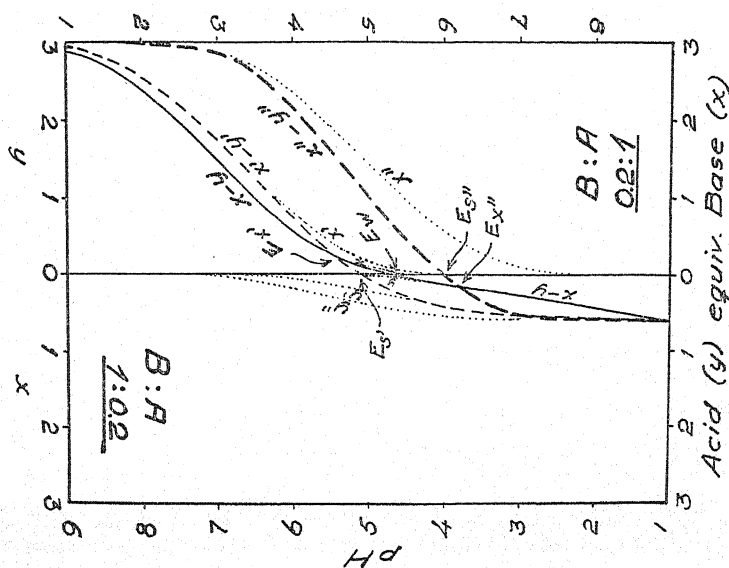


FIG. 82

FIG. 82. THE SAME AS FIGURE 83 WHEN BASOID: ACIDOID = 0.5:1

changing from the dilute to the concentrated solution the point of exchange neutrality $E_{x''}$ occurs far down on the acid side.

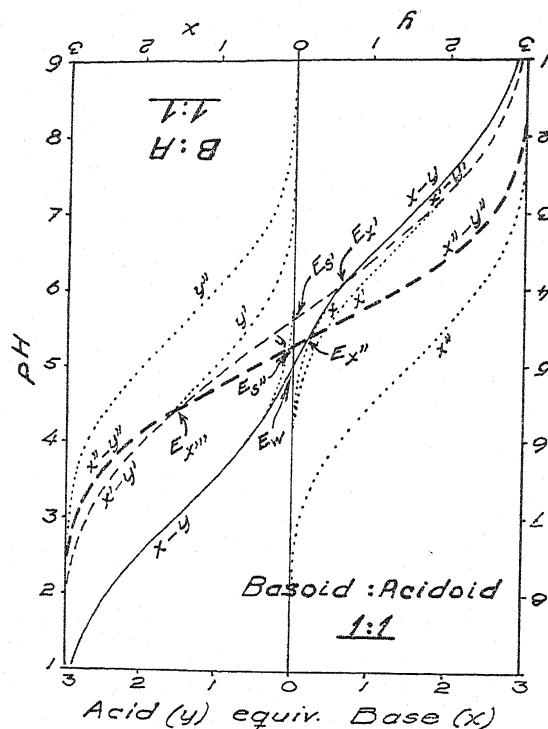


FIG. 83. THEORETICAL CAPACITIES OF A SYSTEM CONTAINING 1 MOL OF AN ACIDOID H_3A AND 1 MOL OF A BASOID $B(OH)_3$ TO BIND BASE AND ACID IN WATER ($x - y$) AND IN A DILUTE ($x' - y'$) AND IN A CONCENTRATED ($x'' - y''$) SOLUTION OF A SALT OF THE TYPE $M^+_2S^-$ WHEN THE APPARENT DISSOCIATION CONSTANTS ARE AS GIVEN IN TABLES 153 AND 153 A

FIG. 83 (INVERTED). THE SAME WHEN THE SALT IS OF THE TYPE $M^{++}S^{--}_2$ AND WHEN THE APPARENT pK VALUES ARE AS GIVEN IN TABLE 153 B

Interpolated, the various amphoteric points in table 153 and in figure 83 are approximately as follows:

	E_w	$E_{s'}$	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$
pH.....	5.0	5.62	5.27	6.10	5.37	4.40

This scattered position of the points of exchange neutrality will give rise to some peculiar and interesting phenomena. Suppose we have a soil possessing the assumed amphoteric properties and suppose that soil to be saturated with base to the extent that its pH lies between the points $E_{x'}$ and $E_{x''}$. A small amount of the salt would then cause an elevation of the pH, whereas a large

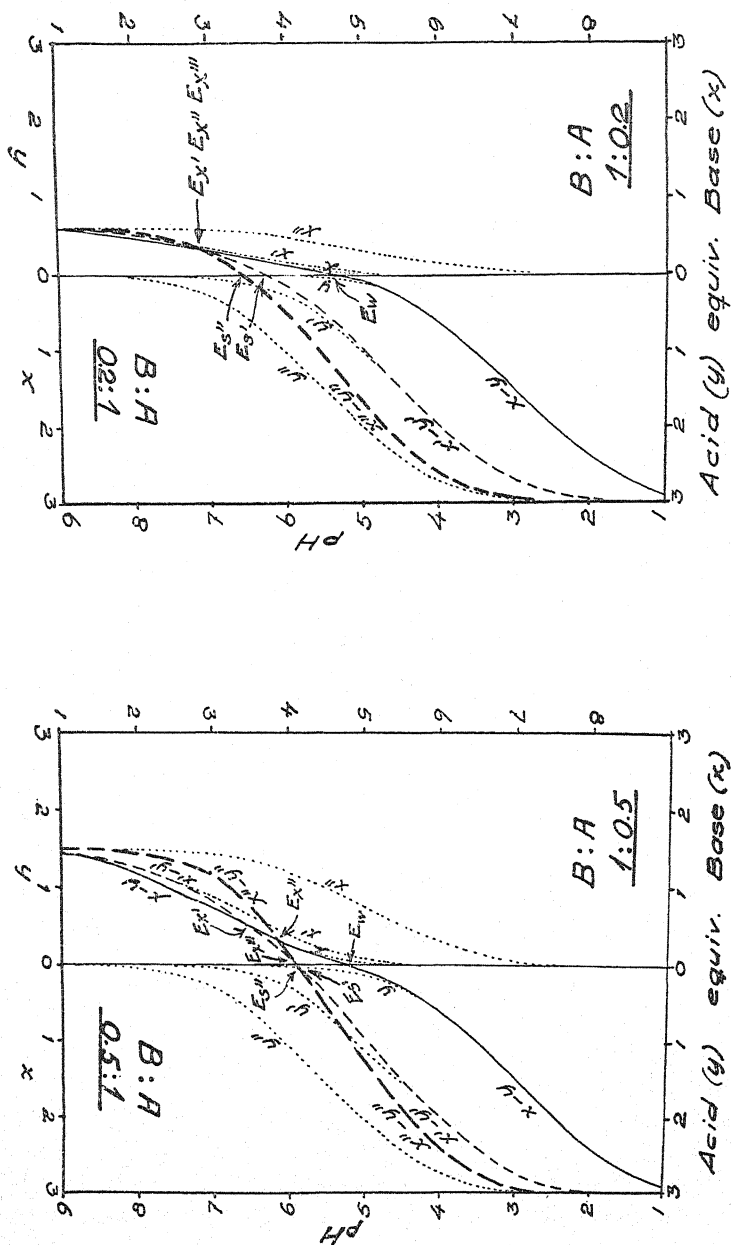


FIG. 85

FIG. 84

FIG. 84. THE SAME AS FIGURE 83 WHEN BASOID: ACIDOID = 1:0.5

FIG. 85. THE SAME AS FIGURE 83 WHEN BASOID: ACIDOID = 1:0.2

amount would cause a lowering of the pH—in the first case an exchange alkalinity, in the second an exchange acidity (and somewhere in between, an exchange neutrality).

We can now study the influence on the aforementioned system of a salt of a divalent cation and a monovalent anion, such as an alkaline earth chloride, merely by turning figure 83 upside down and changing the x values to y values and (in table 153) the plus signs to minus signs, and vice versa. If the pH values are written as indicated in the inverted figure, the k_a and k_b values will be the same, but since the cation is now the ion which has the greatest effect in dilute solution the apparent increase in the constants will be shifted from the one to the other. In terms of $p\bar{k}$, our values for the apparent dissociation constants in water and in solutions of a neutral salt of the type $M^{++}S_2^-$ will now, on the basis of the same assumptions, be those given in table 153 B.

The amphoteric points, interpolated on the inverted figure, have the following approximate values:

	E_w	$E_{s'}$	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$
pH.	5.0	4.38	4.73	3.90	4.63	5.60

The equi-ionic points in the salt solutions and in the points of exchange neutrality have now moved to the opposite side of pH 5.0 ($= E_w$). The in-

TABLE 153 B

Assumed $p\bar{k}$ values in water and in solutions of a neutral salt of the type $M^{++}S_2^-$

	$p\bar{k}_{a_1}$	$p\bar{k}_{a_2}$	$p\bar{k}_{a_3}$	$p\bar{k}_{b_1}$	$p\bar{k}_{b_2}$	$p\bar{k}_{b_3}$
In water.	6.0	7.0	8.0	10.0	11.0	12.0
In dilute solution.	4.5	5.5	6.5	9.75	10.75	11.75
In concentrated solution.	3.5	4.5	5.5	8.0	9.0	10.0

verted figure shows, therefore, why the addition of a neutral salt of the type $M^{++}S_2^-$ to an unsaturated soil, the amphoteric properties of which correspond to our theoretical system, must result in an exchange acidity, and why this acidity must be greater in a dilute than in a concentrated solution of the salt.

We shall now see how changes in the proportions of acidoids to basoids affect the position of the amphoteric points in the different types of salt solutions when the various assumed constants remain the same as those in the system considered in the foregoing discussion.

To do this we need two set of figures for each type of salt, one in which the ratio of acidoid to basoid is increased and one in which it is decreased. But since we can get the effect of one type of salt (e.g., $M^{++}S_2^-$) by turning upside down the figures showing the effect of the other type of salt (e.g., $M^{+}_2S^-$), we can get along with two sets and shall confine ourselves to two figures in each set which, together with figure 83, give us a series of five ratios.

Figures 81, 82, 83, 84, and 85 show the capacities to bind acid and base when the ratios of basoid:acidoid are 0.2:1, 0.5:1, 1:1, 1:0.5, and 1:0.2 respectively, (a) in the upright position when the pk values are as shown in table 153 A, in water ($x - y$) and in a dilute ($x' - y'$) and in a concentrated ($x'' - y''$) solution of a neutral salt of the type $M^{++}S^{-}$, and (b) in the inverted position when the pk values are as shown in table 153 B, when the neutral salt is of the type $M^{++}S^{-}$.

The relationships brought out in the figures will be more easily seen if we put all the amphoteric points together as in table 154.

TABLE 154

The various amphoteric points in figures 81-85 (approximate interpolated pH values)

A. Upright position of figures. pk values as in table 153 A

Neutral salt of the type $M^{++}S^{-}$

FIG.	BASOID: ACIDOID	E_w	$E_{s'}$	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$	$E_w - E_{s'}^*$	$E_w - E_{s''}^*$	$E_{s'} - E_{s''}^*$
81	0.2:1	4.67	5.05	3.97	5.40	3.78	None	-.38	.70	1.08
82	0.5:1	4.93	5.42	4.64	5.82	4.58	3.25	-.49	.29	.78
83	1:1	5.00	5.62	5.27	6.10	5.37	4.40	-.62	-.27	.35
84	1:0.5	5.17	5.85	5.87	6.53	6.12	5.92	-.68	-.70	-.02
85	1:0.2	5.40	6.25	6.55	7.00	7.10	7.10	-.85	-1.15	-.30

B. Inverted position of figures. pk values as in table 153 B

Neutral salt of the type $M^{++}S^{-}$

FIG.	BASOID: ACIDOID	E_w	$E_{s'}$	$E_{s''}$	$E_{x'}$	$E_{x''}$	$E_{x'''}$	$E_w - E_{s'}$	$E_w - E_{s''}$	$E_{s'} - E_{s''}$
81	1:0.2	5.33	4.95	6.03	4.60	6.22	None	.38	-.70	-1.08
82	1:0.5	5.07	4.58	5.36	4.18	5.42	6.75	.49	-.29	-.78
83	1:1	5.00	4.38	4.73	3.90	4.63	5.60	.62	.27	-.35
84	0.5:1	4.83	4.15	4.13	3.47	6.12	4.08	.68	.70	.02
85	0.2:1	4.60	3.75	3.45	3.00	2.90	2.90	.85	1.15	.30

* Negative values = exchange alkalinity. Positive values = exchange acidity.

The values in the $E_w - E_{s'}$ column, table 154, are all negative under A and positive under B. This means that in the unsaturated systems the salt $M^{++}S^{-}$ would, in dilute solution, always give rise to an exchange alkalinity whereas the salt $M^{++}S^{-}$ would, in dilute solution, always yield an exchange acidity. This shows that, as the theory demands, the *valence effect dominates in dilute solutions*.

The values in the $E_w - E_{s''}$ column change from positive to negative (from exchange acidity to exchange alkalinity) as the proportion of the basoid group increases and the acidoid group decreases, and vice versa, in the case of both types of salt. *In concentrated solutions the ratio of acidoids to basoids must therefore be expected to dominate over the valence effect.*

In figure 86 we have made an attempt to show the relationship graphically.

It might seem absurd to plot curves on the basis of so indefinite quantities as "dilute" and "concentrated" solutions, but since we actually do obtain similar curves experimentally and since the figure does bring out the general theoretical relationship when the variables are as above assumed, we find the procedure justified, inasmuch as the curves will give a much better picture than words and tables.

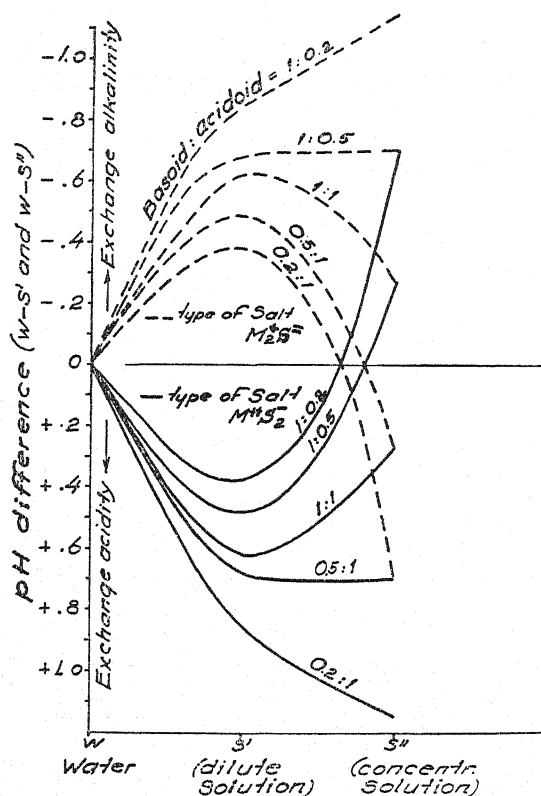


FIG. 86. EXCHANGE ALKALINITY AND EXCHANGE ACIDITY OF THE VARIOUS SYSTEMS DESCRIBED IN TABLES 153-154 AND IN FIGURES 81-85 WHEN ACTED UPON BY "DILUTE" AND "CONCENTRATED" SOLUTIONS OF SALTS OF THE TYPES M_2S_2 AND $M^+S_2^-$

Figure 86 shows that (a) when the group which is acted upon by the divalent ions is in great excess, the deflection of the pH should be greater in a concentrated than in a dilute solution (cf. uppermost and lowest curves in fig. 86; cf. also fig. 85); (b) when the proportion of the acidoid and basoid groups are more balanced the dilute solution should produce the greatest effect (cf. 1:1 ratio curves in fig. 86; cf. also fig. 83); and (c) when the group which is acted upon by the monovalent ions is in great excess the dilute and the concentrated solutions may cause an opposite deflection of the pH (cf. figs. 81 and 82).

A study of figures 81-85 will show that if a soil which contains a higher pro-

portion of acidoids than basoids is saturated to a certain extent with bases, it will give the "regular" type of exchange reaction. No matter which salt we use, we will get an exchange acidity, and this acidity will increase with increasing concentration. The phenomena here discussed must therefore be studied on completely unsaturated soils, and preferably on soils having high equi-ionic points, in order to avoid the disturbing effect of the dissolution of Al and Fe already alluded to.

The foregoing application of the theory illustrates the relationship between the valence effect in the Donnan equilibrium and the ratio of acidoids to basoids in the colloidal complex. We have been discussing this relationship in terms of "dilute" and "concentrated" solutions, but we have said nothing about the degree of dilution or concentration at which the valence effect will become most pronounced. We have said that a dilution favors the entrance of the divalent ions and the outgo of the monovalent ions. But the valence effect operates in the inside as well as in the outside solution. Why then does a dilution favor the entrance of the divalent ions? The question might seem unnecessary, but we are asking it because the answer will pave the approach to the next problem.

When we dilute the system we dilute the outside solution much more than the inside solution because x and y in equation (M) (not to be confused with x and y in the tables and figures in this paper) are reduced virtually in proportion to the dilution, whereas z remains unchanged. It is because the outside solution becomes *relatively* diluted that the equilibrium is deflected toward a greater entrance of divalent ions. The factors which govern this relative dilution are (*a*) the concentration of acidoids and basoids and (*b*) the concentration of the ions dissociated by the colloid. The concentration of the displaced ions in the outside solution as compared to the concentration of the inside solution will be lower (*a*) the lower the concentration of the colloid (in terms of acidoids and basoids) and (*b*) the greater the concentration of the dissociated ions. The more dilute the suspension of a soil and the higher the value of z , the lower must be the concentration at which the valence effect will be most pronounced and the lower, therefore, the concentration at which maxima in exchange acidity and exchange alkalinity will occur.

The approximate relationship is brought out in table 155 and in figure 87. The calculated concentrations (activities) are approximations in so far as we have put the inside concentration equal to a constant ($= z$) and ignored the concentration of the free electrolyte ($= y$ in formula M). This does not involve any serious error as long as the values of x and y are small compared to z , but where the outside concentration (x) approaches that of the inside ($y + z$), as in II a, table 155 and figure 87, the results become misleading. With this exception the results are sufficiently accurate to bring out the true relationship.

The calculations apply to an unsaturated amphoteric soil of which the acidoid/basoid ratio $= 1$ and which is assumed to be present in the concen-

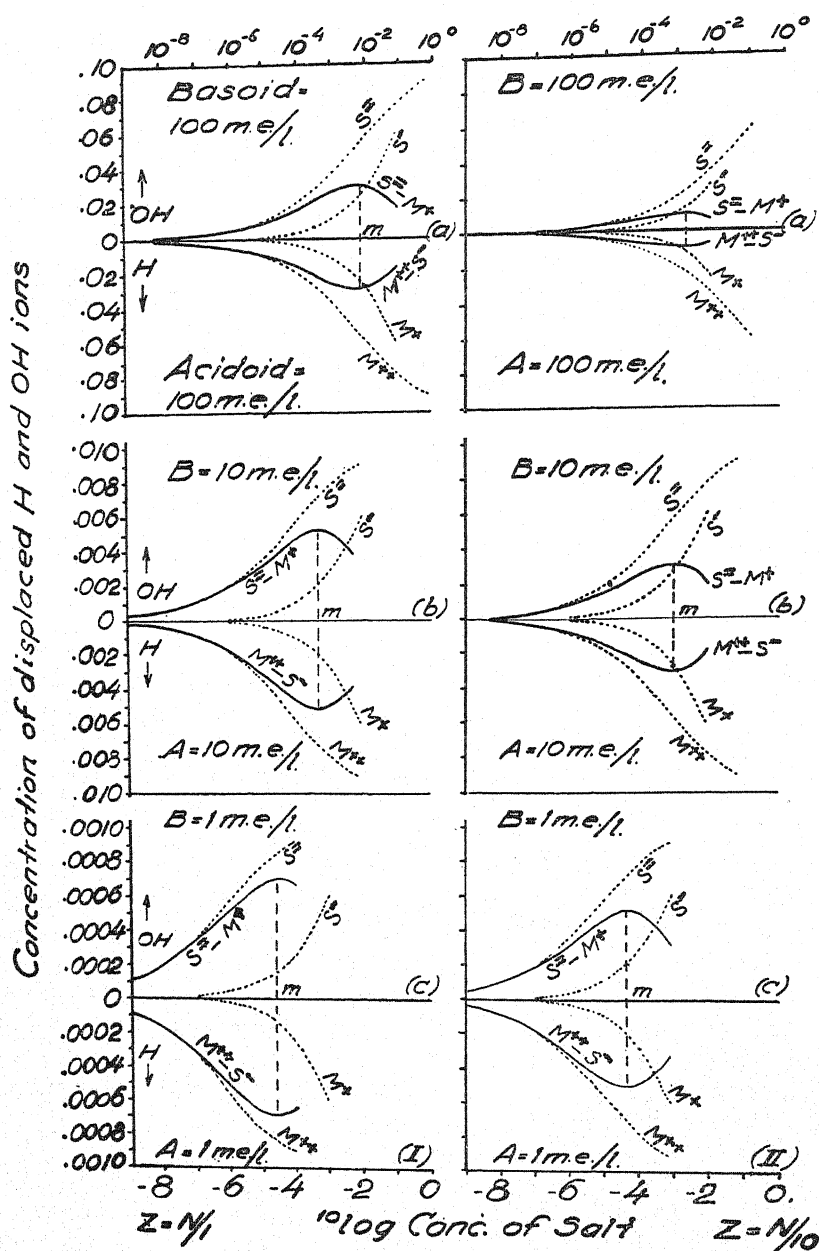


FIG. 87. THE MAXIMA IN EXCHANGE ALKALINITY AND EXCHANGE ACIDITY IN SOLUTIONS OF SALTS OF THE TYPES M^+S^- AND $\text{M}^{++}\text{S}^{2-}$ AS RELATED TO THE CONCENTRATION OF COLLOID (a, b, AND c) AND TO THE CONCENTRATION (z) OF THE IONS DISSOCIATED BY THE COLLOID (I AND II) AS BASED ON TABLE 155

TABLE 155

Approximate theoretical displacement of H and OH ions at various concentrations of an unsaturated soil [acidoid = basoid = (a) 100, (b) 10, and (c) 1 m.e. per liter] by neutral salts of the type $M^{++}S_2^-$ and $M_2^+S^-$ when the activity (z) of the ions dissociated by the colloid is (I) 1N and (II) 0.1N [cf. formula (I) and (J) and fig. 87].

ACTIVITY IN THE INSIDE SOLUTION:		ACTIVITY IN THE OUTSIDE SOLUTION:		
H ⁺ or OH ⁻	M ⁺ or M ⁺⁺ S ⁻ or S ²⁻	H ⁺ or OH ⁻	M ⁺ or S ⁻	M ⁺⁺ or S ²⁻
N	N	N	N	N
(I a) = 100 m.e./l., $z = 1N$				
.9	.1	.01	.0011	1.23×10^{-5}
.8	.2	.02	.0050	1.25×10^{-4}
.6	.4	.04	.0266	.00178
.5	.5	.05	.0500	.005
.4	.6	.06	.0900	.0135
.2	.8	.08	.3200	.128
.1	.9	.09	.8100	.729
(I b) = 10 m.e./l., $z = 1N$				
.9	.1	.001	.00011	1.23×10^{-7}
.8	.2	.002	.00050	1.25×10^{-6}
etc.
(I c) = 1 m.e./l., $z = 1N$				
.9	.1	.0001	.000011	1.23×10^{-9}
.8	.2	.0002	.000050	1.25×10^{-8}
etc.
(II a) = 100 m.e./l., $z = 0.1N$				
.09	.01	.01	.0011	1.23×10^{-4}
.08	.02	.02	.0050	1.25×10^{-3}
etc.
(II b) = 10 m.e./l., $z = 0.1N$				
.09	.01	.001	.00011	1.23×10^{-6}
.08	.02	.002	.00050	1.25×10^{-5}
etc.
(II c) = 1 m.e./l., $z = 0.1N$				
.09	.01	.0001	.000011	1.23×10^{-8}
.08	.02	.0002	.000050	1.25×10^{-7}
etc.

trations of 100, 10, and 1 m.e. per liter, the value of z being in one case (I) put equal to 1 N and in the other (II) equal to 0.1 N.

The assumption of a complete dissociation of the acidoid and basoid as well

as of their saloids makes the concentration of the displaced H and OH ions very large. But this need not disturb us, for we are here not concerned with the true concentrations of the displaced H and OH ions but with the relative proportions of the displaced ions.

Table 155 and figure 87 show that the maxima (m) in exchange acidity and exchange alkalinity must occur at a lower concentration of the salt (a) the lower the concentration of the soil suspension (or the lower the colloid content of the soil) and (b) the higher the concentration (z) of the ions dissociated by the colloid (II a omitted).

The maxima in figure 87 occur approximately at the following concentrations of the salts $M^+_2S^-$ and $M^{++}S^{--}_2$:

ACIDOID = BASOID =	100	10	1 M.E./L.
$z = 1N$.008	.00045	.000025
$z = 0.1N$0009	.00005

These positions of the maxima, especially those at the higher concentrations, are somewhat lower than the true theoretical positions, because of the use of z instead $y + z$.

The two maxima in each pair of curves in figure 87 occur at the same concentration of salt because in each system we are assuming an equivalence between acidoids and basoids and assign the same value for z to both groups. In the soil we might have any proportion between these factors, and the maxima in exchange acidity and exchange alkalinity may, therefore, occur at different concentrations of the salts. If, for example, the basoid content of a system be decreased, or the acidoid content increased, then the maximum in exchange acidity will not only become greater but must also be deflected to a higher salt concentration, whereas the maximum in exchange alkalinity will become smaller and occur at a lower concentration of salt. If, on the other hand, the acidoid content of the system be decreased, or the basoid content increased, then the effect will be the opposite.

This is illustrated in figure 88. The calculations apply to unsaturated soil suspensions whose acidoid/basoid ratios vary between 5.0 and 0.2 and whose acidoid and basoid concentrations are put at 2 to 10 m.e. per liter, the value of z being assumed to be equal to 1 N in every case.

If we compare the curves in figure 88 with the curves in I b in figure 87 we note that a decrease in basoids (= increase in acidoid/basoid ratio) causes the maximum in exchange alkalinity (m^-) to be smaller and to be deflected toward a lower salt concentration, whereas the maximum in exchange acidity (m^+) becomes larger and is deflected toward a higher concentration of salt (cf. curves in fig. 88 a and b). A decrease in acidoids (= decrease in acidoid/basoid ratio) has the opposite effect (cf. curves in fig. 88 c and d).

The maxima in exchange alkalinity in solution $M^+_2S^-$ and in exchange

acidity in solution $M^{++}S_2^-$ occur, in the different proportions of acidoid to basoid, approximately at the following equilibrium concentrations of the salts:

FIGURE.....	88 b	88 a	87 I b	88 c	88 d
Acidoid.....m.e./l.	10	10	10	5	2
Basoid.....m.e./l.	2	5	10	10	10
$M^{++}S_2^-$N	.000016	.00010	.00045	.00135	∞
$M^{++}S_2^-$N	∞	.00135	.00045	.00010	.000016

In part B, we shall present our experimental data and show how we have applied the principles to a study of soil profiles.

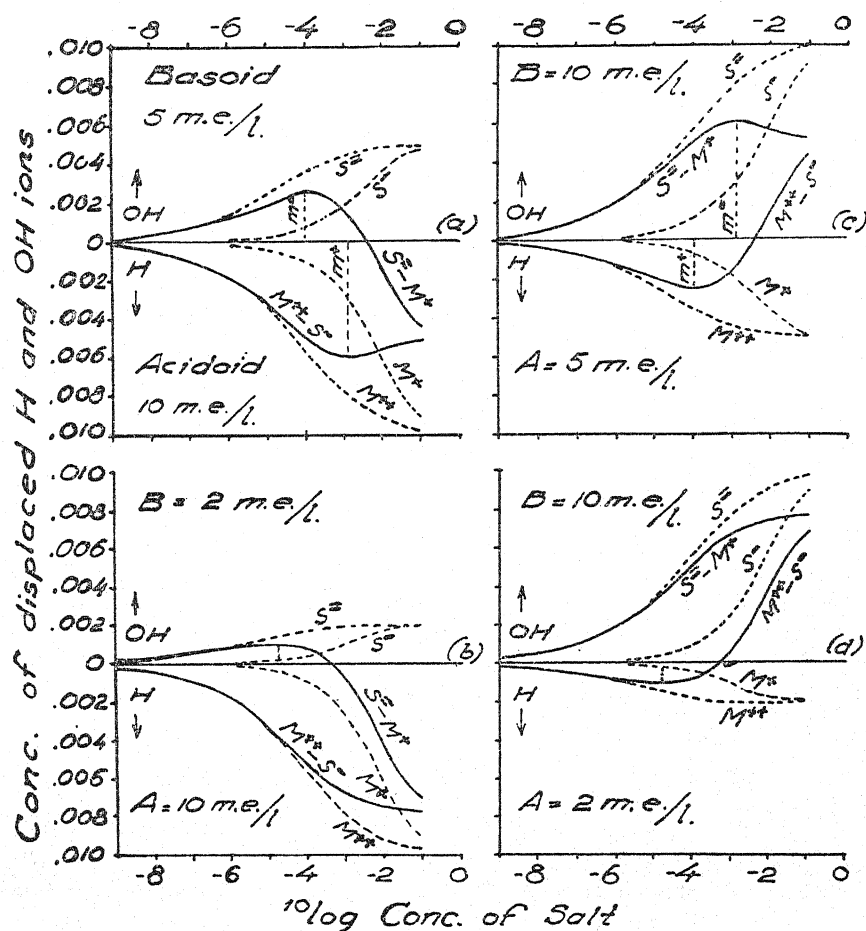


FIG. 88. THE MAXIMA IN EXCHANGE ALKALINITY AND EXCHANGE ACIDITY AS RELATED TO THE ACIDOID/BASOID RATIO

REFERENCES

- (1) DU RIETZ, C. 1938 Über das Ionenbindungsvermögen fester Stoffe. Dissertation, Kgl. Tekn. Högskolan, Stockholm.
- (2) MATTSON, S. 1929 The laws of soil colloidal behavior: I. *Soil Sci.* 28: 179-220.
- (3) MATTSON, S. 1932 The laws of soil colloidal behavior: VIII. Forms and function of water. *Soil Sci.* 33: 301-322.
- (4) MATTSON, S., AND HOU, KWANG-CHIUNG. 1937 The laws of soil colloidal behavior: XX. The neutral salt effect and the amphoteric points of soils. *Soil Sci.* 44: 151-166.
- (5) MATTSON, S., AND WIKLANDER, L. 1937 The equi-ionic point and the point of exchange neutrality of soils. *Ann. Agr. Col. Sweden* 4: 169-189.
- (6) MATTSON, S., AND KARLSSON, N. 1938 The electrochemistry of soil formation: II. The phosphate complex. *Ann. Agr. Col. Sweden* 6: 109-157.
- (7) MÖLLER, J. 1935 Studier over Ionbytningsprocessen, med saerlig Henblik paa Agrikulturmekien. Dissertation, Danmarks Tek. Højskole, Kopenkagen.
- (8) SCHOFIELD, R. K. 1935 The interpenetration of the diffuse double layer surrounding soil particles. *Trans. Third Internat. Cong. Soil Sci.* 1: 30-33.
- (9) TERÄSVUORI, A. 1930 Über die Bodenazidität, mit besonderer Berücksichtigung des Elektrolytgehaltes der Bodenaufschlämmungen. Akad. Abhandl., Helsinki (Valtion Maatalouskoet. Julkaisu. 29).

THE LAWS OF SOIL COLLOIDAL BEHAVIOR: XXI B. THE AMPHOTERIC POINTS, THE pH, AND THE DONNAN EQUILIBRIUM

PART B. EXPERIMENTAL

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MATERIALS USED

For a systematic study of the application of the principles elucidated in part A to amphoteric soils, the following materials were selected:

1. Soil and three subsoils of a ferruginous laterite from Las Mesas plateau, Mayaguez, Puerto Rico. This is the same soil which was previously studied by Mattson and Gustafsson (3) and which was kindly sent us by Dr. J. A. Bonnet, chief of the Division of Soils at the Insular Experiment Station, Rio Piedras. The four samples, which we shall call "Las Mesas," were taken at the following depths: I = 0-12 inches, II = 13-23 inches, III = 26-38 inches, and IV = 43-58 inches. Only samples I and IV have been titrated.

2. Selected samples from a collection of 190 from a hydrologic podzol series of profiles taken in a trench 5 m. long which was dug from a wet depression into an adjoining hill of fine sandy material covering every transition from a peat podzol to the dry type of iron podzol (plate 4). This series which is taken near the south end of Lake Unden in Västergötland is the object of an extensive investigation by Mattson and Lönnemark (4). The profile series is expressed in the form of coordinate values of x and y , $x = 0$ cm. representing the wet end and $x = 500$ cm. the dry end of the series, and y the depth of the sample below a horizontal line originating at the surface of the ground at the upper (dry) end of the series as shown in plate 4.

For our present work we selected three of the fifteen profiles included in the work of Mattson and Lönnemark, viz., $x = 0$, $x = 280$, and $x = 500$ cm. (cf. fig. 99). From these three profiles we selected a horizontal series by taking the most strongly basoid sample in the B horizon of each profile (cf. fig. 101). The titrations, which required a considerable amount of material, had to be made on a collection of large samples from a separate profile dug at a point $x = 400$ cm. The following samples were titrated: $B_2 = 27-32$ cm., $B_3 = 37-42$ cm., $B_4 = 50-55$ cm., and $B_5 = 80-85$ cm. (cf. fig. 92-96).

Previous attempts to study the pH of laterites in different solutions by the quinhydrone and hydrogen electrodes have been unsuccessful, but the use of a glass electrode has apparently removed all difficulties. The laterites are particularly suitable for this kind of study, because of their high equi-ionic points which allow an extensive adsorption of anions before any appreciable dissolution of Al and Fe takes place. (Such dissolution causes the exchange acidity of a strong solution to appear greater than that of a dilute solution even where the reverse is true.)

Most laterites and all samples from the podzol B horizon are virtually completely unsaturated and need not be electrodyalized. The Las Mesas subsoil contained practically no dialyzable bases, but a not inappreciable quantity of SO_4 was present. This anion is held so firmly that it becomes dialyzable only after the soil is made alkaline by ammonia. In the following experiment all samples were used in their natural condition.

TITRATION CURVES AND THE AMPHOTERIC POINTS OF SOILS

Figures 89 and 90 give the titration curves of the Las Mesas I and IV in water and in 1 *N* and 0.01 *N* Na_2SO_4 . Without an application of the mass law and the valence effect as expressed by the Donnan equation, these curves would, with respect to their intersections and relative position, be inexplicable. But on the basis of the theory, as here developed, the explanation presents itself in the form of an almost perfect reproduction of the theoretical curves. Since the theoretical curves were constructed before the titrations were carried out, we were surprised to find so good an agreement between theory and experiment.

A comparison of figure 89 with figure 83 (see part A) and of figure 90 with figure 85 (see part A) leaves no doubt as to the meaning of the relative position of the experimental curves: In Las Mesas I (surface soil) the activities of the acidoid and basoid groups are more nearly balanced, because of the presence of humus acidoids and the less active (more dehydrated and aged) sesquioxides, whereas in Las Mesas IV the activity of the basoid groups is far in excess. The result is that, in Las Mesas I, the exchange alkalinity in the 0.01 *N* Na_2SO_4 solution is greater than that in the 1 *N* solution, whereas in Las Mesas IV the exchange alkalinity, which is very great, increases with increasing concentration.

Because of its strong basoid character the Las Mesas IV ought to show the valence effect by yielding a greater exchange acidity in a dilute than in a concentrated solution of a salt of the type $\text{M}^{++}\text{S}^{--}_2$. A preliminary experiment with BaCl_2 (cf. fig. 97, 0 per cent humus) gave a maximum exchange acidity in a 0.1 *N* solution. The Las Mesas IV was therefore titrated in water and in 0.1 and 1 *N* BaCl_2 , with the results shown in figure 91.

It will be noted that figure 91 is an approximate counterpart to figure 83 in the inverted position. This points to an approximate equivalence between the acidoids and basoids, whereas the reaction with the sulfate points to a great excess of basoids. On the basis of the behavior of the soil in the sulfate solution we should expect figure 91 to resemble figure 81 in the inverted position (see part A), that is, the Las Mesas IV ought to yield an exchange acidity in the dilute and an exchange alkalinity in the concentrated BaCl_2 solution.

This anomaly in behavior we ascribe to the SO_4 ion which is present in the exchangeable form to the extent of 4.6 m.e. per 100 gm. These ions are, of course, precipitated by the Ba ions, leading to a substitution of SO_4 by Cl ions. The monovalent Cl ions have a lower displacing power than the SO_4

ions. The place of the latter in the basoid complex will therefore be taken by OH as well as by Cl ions, and this leads to an exchange acidity, produced by an extramolecular reaction.

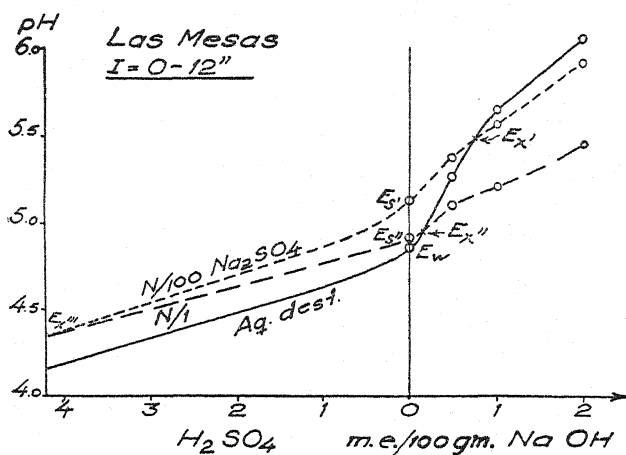


FIG. 89

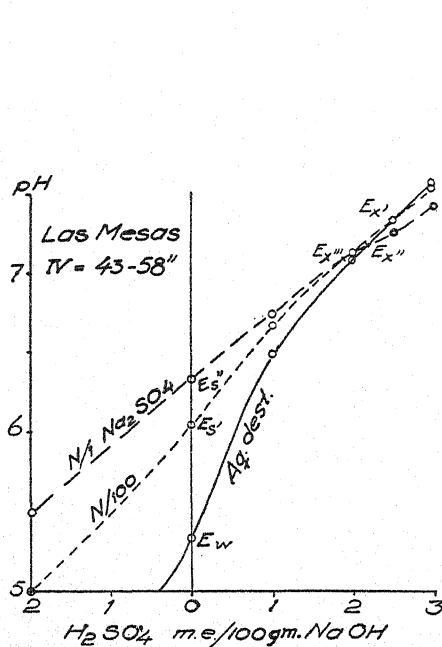


FIG. 90

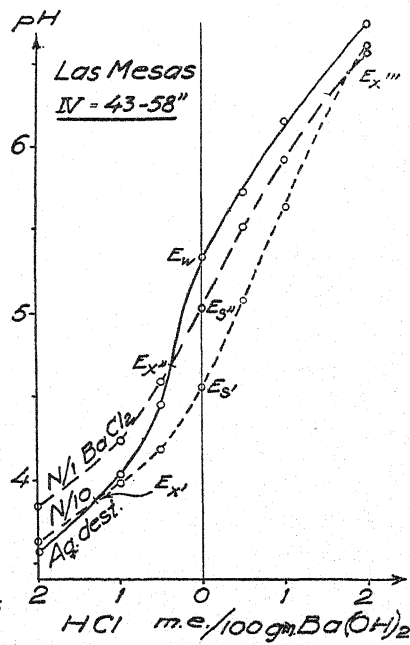


FIG. 91

FIGS. 89, 90, AND 91. TITRATION OF THE LAS MESAS LATERITE

The presence of SO_4 in the laterite demands the use of CaCl_2 instead of BaCl_2 if this extramolecular reaction is to be avoided. But we had obtained better

maxima in exchange acidity with Ba than with Ca ions and since the laterite yielded a pronounced maximum in BaCl_2 (0.1 *N*) we decided to use this salt even for this soil. We find the anomaly instructive, and later (cf. fig. 101) we shall show evidence in support of our interpretation.

The amphoteric points of the three laterite samples in the solutions employed are as follows:

FIGURE	SAMPLE	SALT	E_w	E_s'	E_s''	E_x'	E_x''	E_x'''
89	Las Mesas I	Na_2SO_4	4.86	5.12	4.90	5.47	4.94	4.35
90	Las Mesas IV	Na_2SO_4	5.33	6.04	6.33	7.37	7.16	7.08
91	Las Mesas IV	BaCl_2	5.33	4.55	5.03	3.87	4.68	6.43

It is interesting to note that the points of exchange neutrality of Las Mesas IV in Na_2SO_4 all occur above pH 7.

Figures 92 to 95 show the titration in water and in 0.01 and 1 *N* Na_2SO_4 of the B_2 , B_3 , B_4 , and B_5 samples of the Udden podzol ($x = 400$).

It will be noted that the activity of the basoid group decreases and that of the acidoid group increases (at least relatively) as we go from the B_2 to the B_5 horizon. Thus, the B_2 sample yields an exchange alkalinity (in the unsaturated condition) in the 1 *N* as well as in the 0.01 *N* solution, the B_3 sample yields an exchange alkalinity only in the 0.01 *N* solution, and the B_4 and B_5 samples yield exchange acidity in both concentrations of the salt.

A comparison with the theoretical curves (see part A) will show that figure 92 resembles figure 83 and that figure 93 resembles figure 82 with respect to the relative position of the amphoteric points. The titration curves of B_4 and B_5 have no theoretical counterpart among figures 81 to 85. From the large amount of exchange alkalinity developed on the acid side of the points of exchange neutrality, we know that these samples possess a considerable quantity of basoids. Why then does not the valence effect show itself here as in the case of the other samples by yielding an exchange alkalinity in the "dilute" solution?

The answer to this question is, as will be shown later, that the "dilute" solution was not dilute enough. The maximum effect of the valence occurs here, where the basoid groups are relatively weak, at a lower concentration than 0.01 *N* Na_2SO_4 (cf. fig. 96).

The amphoteric points of the four podzol samples in the solutions employed are as follows:

FIGURE	SAMPLE	E_w	E_s'	E_s''	E_x'	E_x''	E_x'''
92	B_2	5.01	5.41	5.15	7.71	5.46	4.93
93	B_3	4.91	5.13	4.79	5.50	4.75	4.68
94	B_4	4.70	4.65	4.45	4.55	4.39	4.38
95	B_5	4.70	4.50	4.25	4.15	4.15	4.15

A great deal of work has been done to determine the various factors which influence the pH of the soil, such as dilution and salt concentration. In view

of the foregoing relationships, we can readily appreciate why so little progress has been made. It is obvious that the soil reaction is so intimately related

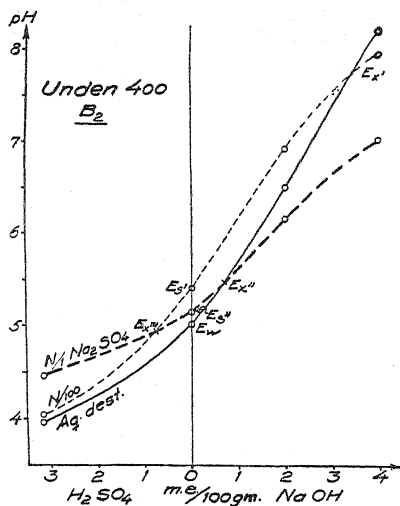


FIG. 92

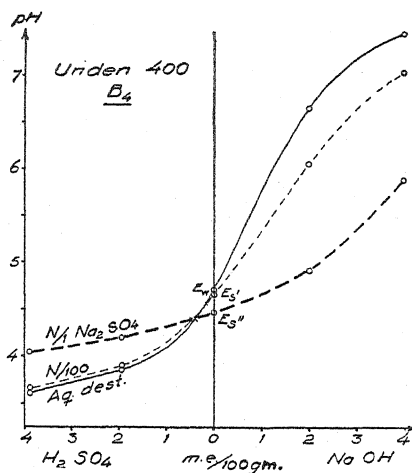


FIG. 94

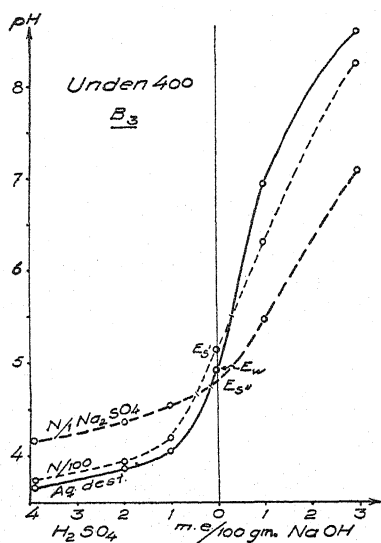


FIG. 93

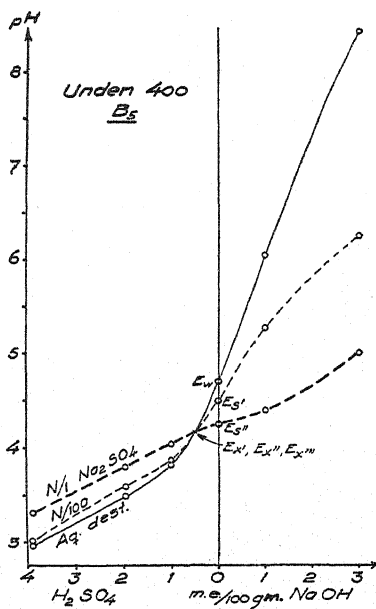


FIG. 95

FIGS. 92, 93, 94, AND 95. TITRATION OF THE B SAMPLES OF THE UNDEN PODZOL PROFILE 400.

to the amphoteric nature of the soil complex and to the Donnan equilibrium that no student who does not embrace these ideas can hope to solve its prob-

lems. The fact that some of the E_s values are higher and some lower than the E_w values and that the E_x values vary between 4.15 and 7.71 in samples from the same soil profile could never be accounted for without the theory as here presented. The attempt by Mattson and Gustafsson to relate the point of exchange neutrality to the point of zero, net base saturation (base-forming cations minus acid anions) will apparently fail, since these points are variables related to other, indeterminable variables.

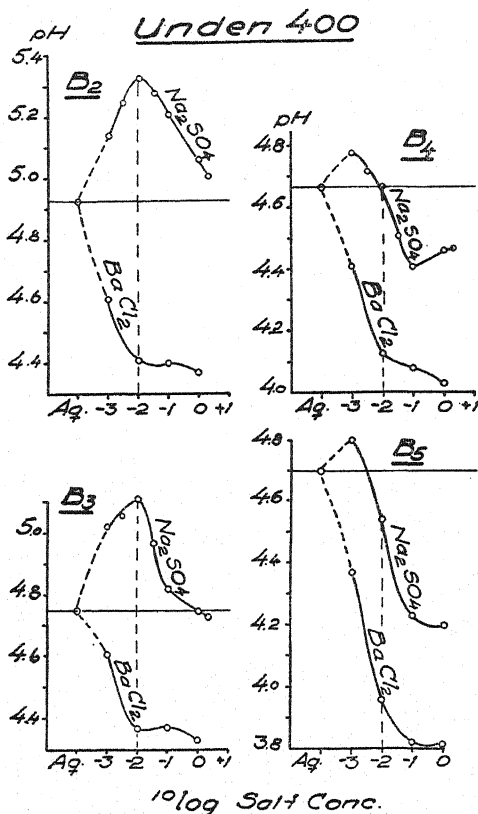


FIG. 96. THE MAXIMA IN EXCHANGE ALKALINITY AND EXCHANGE ACIDITY IN THE B HORIZONS OF THE UNDEN PROFILE 400

THE MAXIMA IN EXCHANGE ACIDITY AND ALKALINITY

Figure 96 gives the pH in water and in various concentrations of BaCl₂ and Na₂SO₄ of the four Unden podzol samples shown in figures 92–95.

The following facts are of interest in connection with the theory:

1. The exchange alkalinity in Na₂SO₄, which is greatest in B₂, decreases with an increase in exchange acidity in BaCl₂, which is greatest in B₅.
2. As the exchange alkalinity is decreased, its maximum is deflected toward a lower concentration of salt (from an initial concentration of about 0.01 *N* in B₂ to 0.001 *N*, or

less, in B_3). At the same time the maximum in exchange acidity is deflected toward a higher concentration of salt.

This is in agreement with the theory, as illustrated in figure 87 I b and figure 88 a and b (see part A). It is obvious that when the acidoids greatly exceed the basoids the exchange alkalinity must reach a vanishing point at very low concentration of salt, and that the exchange acidity must assume a maximum in the most concentrated solution, exactly as shown in the figures.

We can now understand why we found no theoretical counterpart to figures 94 and 95. Had we titrated the B_4 and B_5 samples in 0.001 N instead of 0.01 N Na_2SO_4 we would have obtained a relative position of the curves similar to those in figure 81. The "dilute" solution in figures 94 and 95 was not dilute enough to show a maximum of the valence effect.

We are unable to explain the decrease in exchange acidity of B_4 in Na_2SO_4 above 0.1 N unless it can be ascribed to abnormal changes in the activity coefficients of the various ions at high concentrations in the inside solution. (An abnormal decrease in the activity coefficient of the SO_4 ions or an increase of that of the Na ions in the inside solution would account for the phenomenon.)

Influence of humus

The difference in amphoteric behavior between Las Mesas I and IV is to a large extent ascribed to humus acidoids. A systematic study of the influence of humus upon the reactions of the laterite was made by adding 2.5, 5.0, and 10.0 per cent of humus acidoid (obtained by extracting peat with NaOH, precipitating with H_2SO_4 , and electro dialyzing the precipitate until free from acid) to the Las Mesas IV and then determining the pH in water and in increasing concentrations of Na_2SO_4 and $BaCl_2$. The results are shown in figure 97. In comparison with the sample to which no humus was added, we note the following:

1. The pH in water is lowered.
2. The exchange alkalinity in Na_2SO_4 is reduced, and this reduction is much greater in the concentrated (1 N) solution, which yields an exchange acidity with 5 and 10 per cent humus. This gives rise to pronounced maxima in exchange alkalinity in the dilute solutions (between 0.01 and 0.1 N initial concentration).
3. The exchange acidity in $BaCl_2$ is increased, and this increase is greater in the concentrated solution (1 N); the maxima in dilute solutions (about 0.1 N), therefore, become less pronounced and finally vanish (with 10 per cent humus).

That this is all in agreement with the theory is best shown in figure 98, in which the exchange alkalinities and exchange acidities are plotted against the salt concentration in analogy to figure 86 (see part A).

It will be noted that the curves in figure 98 are "deformed" toward the lower right side. This is doubtless, in part, due to the SO_4 ions present in the laterite. The precipitation of these ions as $BaSO_4$ leads to an additional exchange acidity, as has been explained. This causes the maximum in exchange acidity to occur at a higher concentration of salt (about 0.1 N) than would be

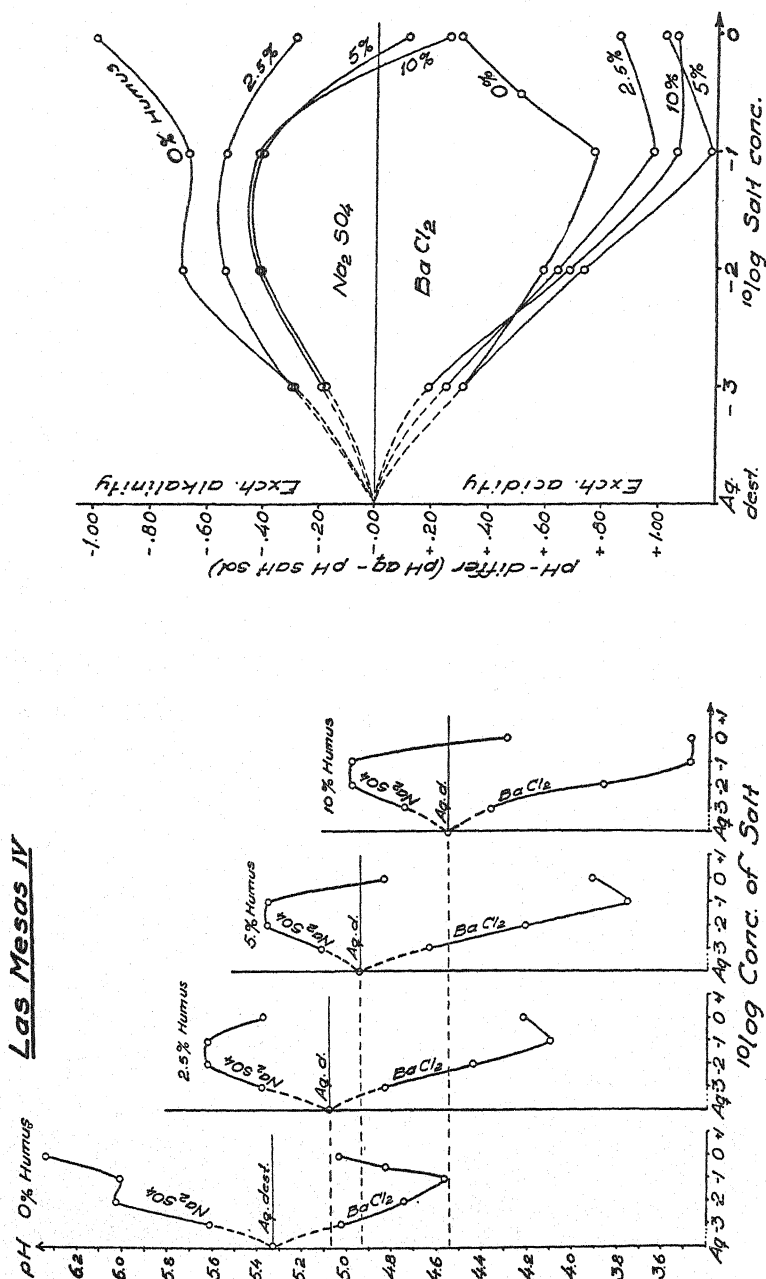


FIG. 97

FIG. 97. INFLUENCE OF HUMUS ON THE EXCHANGE REACTIONS OF THE LAS MESAS LATERITE SUBSOIL

FIG. 98. THE SAME AS FIGURE 97, PRESENTED IN ANALOGY TO FIGURE 86

FIG. 98

the case in the absence of the SO_4 ions. The "deformation" of the curves may also, in part, be an expression of the aforementioned dissolution and hydrolysis of the compound formed by the anions with the basoid groups, thus changing the exchange alkalinity to an exchange acidity. In laterites having high equi-ionic points this effect is small.

It should be pointed out that, since our salt concentrations express the initial (not the end) concentrations, the curves do not represent the exact equilibrium conditions. The maxima occur in reality at a somewhat lower concentration than that shown in the figures. Since this deviation is different for the different ions as well as for differences in the composition of the complex, this will also affect the form and relative position of the curves. A comparison of figures 86 and 98 leaves, nevertheless, no doubt as to the application of the theory.

It should also be pointed out that the maxima in figure 98 should, in reality, not occur at the same concentration of salt as in figure 86, which is based on a single "dilute" concentration, but should be deflected, as explained in connection with figures 88 and 96. The fact that this deflection is not in evidence in figure 97, with respect to the maxima in exchange alkalinity, leads us to add a few more words concerning this experiment.

In figures 97 and 98 there is no reduction in the exchange alkalinity beyond the two first additions of humus (2.5 and 5 per cent), and there is no visible deflection of these maxima in the direction of a lower concentration of salt.

We consider this to be significant and to be an expression of the condition of the mixture. The humus acidoid was added to the soil in the form of a gel. It seems obvious that, in spite of the shaking, the relatively large particles of the humus could not enter the smallest capillaries of the laterite but could merely interact with the basoid groups situated on the surface. Only the first additions of humus, which "neutralized" these outer groups, had, therefore, any effect on the basoid activity. The basoid groups in the interior were left free to interact with the SO_4 ions. It is obviously possible for a system to contain free acidoids and basoid groups of any strength as long as these groups are prevented from interacting. This fact may have important applications, because it is theoretically possible to remove virtually all the anions and cations from a solution by passing it alternately through an acidoid and a basoid.

If, in the foregoing experiment, we had used Na-humate instead of the humus acidoid (and electrodialyzed the mixture) we would undoubtedly have obtained a different picture, although the acidoid/basoid *mass* ratio would have been the same.

Besides the vertical series from the B horizons of the Unden podzol profile $x = 400$, we selected, as has already been mentioned, a horizontal series by taking the most strongly basoid sample (cf. fig. 101) from three of the Unden series of profiles.

Figure 99 gives the pH in water and in different concentrations of Na_2SO_4 and BaCl_2 . Sample $x = 500$ ($y = 30$) comes from the dry end of the profile

series and is the most strongly basoid of all. With a loss on ignition of only 1.47 per cent, this sample yields an exchange alkalinity with all but the dilute solutions of BaCl_2 . The nearest theoretical counterpart is found in figure 88 d (see part A).

Sample $x = 280$ ($y = 90$), with its 2.03 per cent loss on ignition, is somewhat less strongly basoid, but even this sample yields a slight exchange alkalinity in N BaCl_2 . The nearest theoretical counterpart is found in figure 88 c (see part A).

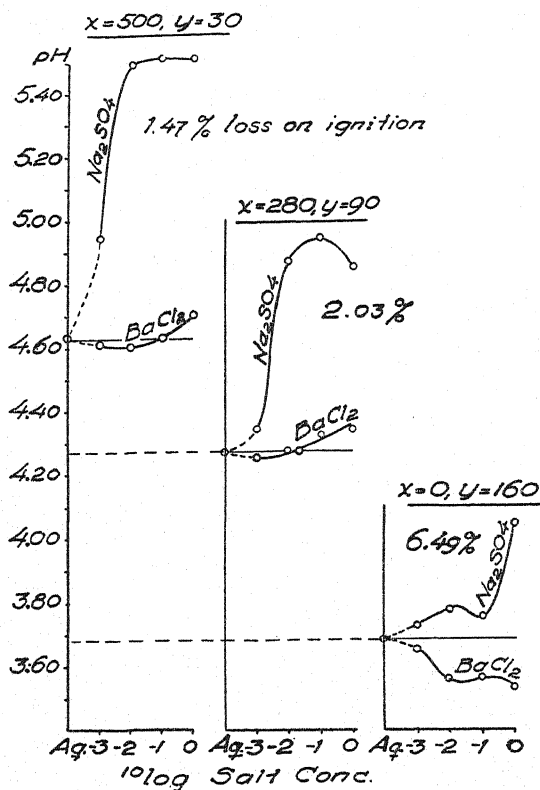


FIG. 99. EXCHANGE REACTIONS OF THE MOST BASOID SAMPLES FROM THREE OF THE UNDEN PROFILES
(Cf. fig. 101)

Sample $x = 0$ ($y = 160$), with 6.49 per cent loss on ignition, yields curves which seem difficult to interpret. It should be pointed out that this sample comes from the wet end of the profile series and represents apparently gleyed material. The low pH might be another factor. The sample apparently possesses a fairly strong basoid group in spite of the high humus content, but before the samples are analyzed it is impossible to say what specific influence may be present.

Influence of the concentration of the colloid

Having shown how variations in the acidoid/basoid activity ratio affect the maxima in exchange acidity and exchange alkalinity, we now show how variations in the concentration of the colloid affect these maxima when the acidoid/basoid ratio remains the same.

According to figure 87 (see part A) an increase in concentration should deflect the maxima toward a higher concentration of salt. Figure 100 shows the

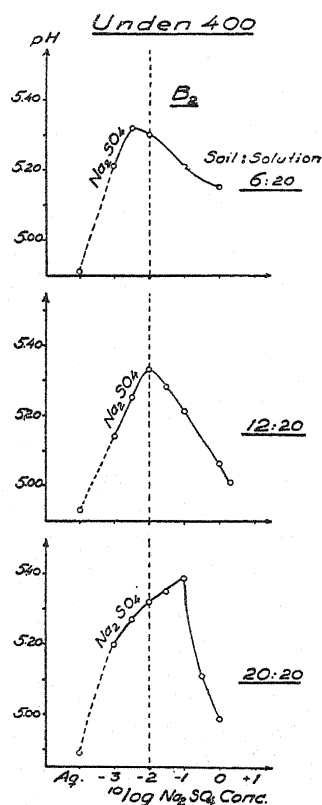


FIG. 100. INFLUENCE OF THE CONCENTRATION OF THE SOIL SUSPENSION ON THE MAXIMUM IN EXCHANGE ALKALINITY

exchange alkalinities in various concentrations of Na_2SO_4 of the B_2 sample of the Unden profile 400 in the proportions of 6, 12, and 20 gm. of soil to 20 cc. solution.

The curves show that an increase in the concentration of the soil results in an increase in exchange alkalinity and a deflection of the maximum toward a higher concentration of salt.

We note further that the right legs of the curves become steeper with increasing amounts of soil. This is because the counter action (exchange

acidity) of the Na ions is less effective in the dilute suspension, as dilution opposes the lowering and favors the elevation of the pH.

The maximum in exchange alkalinity in a dilute suspension should be smaller than the exchange alkalinity of a more concentrated suspension at the corresponding concentration of the salt solution. This is true for the 12:20-20:20 pair but, for some reason, not for the 6:20-12:20 pair of systems.

With this exception, the results are in perfect agreement with the theory and yield information which, if it could be quantitatively interpreted, would be of very great importance, for we should then be able to determine the quantity of colloid in a soil merely by means of a few simple pH determinations. But even the qualitative results which the method yields will lend themselves to important applications.

APPLICATION TO SOIL PROFILES

Having established the factors which govern the amphoteric reactions of soils, we are in a position to reciprocate and, by means of these reactions, i.e., the pH, determine qualitatively and, in a comparative sense, quantitatively, these factors.

The results of the application of the method to three of the Unden profiles and to the Las Mesas laterite are here presented.

Figure 101 gives the difference between the pH of the soil in water and its pH in 0.01 N Na_2SO_4 and $BaCl_2$ solutions at different depths of the profiles. These differences express the exchange acidity (positive values) and the exchange alkalinity (negative values) of the soil materials.

Since the samples have not yet been analyzed, it is too early to attempt a detailed discussion of the results. In the case of the podzol profiles we shall merely direct the reader's attention to the maximum in exchange alkalinity in the upper B horizon. This maximum expresses a high basoid activity resulting from the isoelectric precipitation of the cationic sol complex which has come from the A horizon. Then there is another maximum in the gley horizon (G) due to an accumulation of ferric hydroxide formed by the oxidation of soluble ferrous compounds.

This method, which is based upon a determination of the *activities* of the acidoid and basoid groups (as distinguished from the analytical determination of the *quantities* of acidoid and basoid materials), offers a simple, direct, and scientific expression for the podzolization of a soil. [For a complete report on the work on the Unden series cf. Mattson and Lönnemark (4).]

The samples of the laterite used in this experiment were electrodyalyzed to remove the displaceable SO_4 ions which were present in small but appreciable amounts in the II, III, and IV samples. Sample I contained very little SO_4 but contained, on the other hand, appreciable quantities of Ca ions, of which the other samples contained mere traces.

The pH values of the electrodyalyzed samples (the ultimate pH) in water and in 0.01 N and N Na_2SO_4 and $BaCl_2$ are given in table 156. These values

represent the average of duplicate determinations with the glass electrode, which gave very good results. On the basis of these values, the basoid ac-

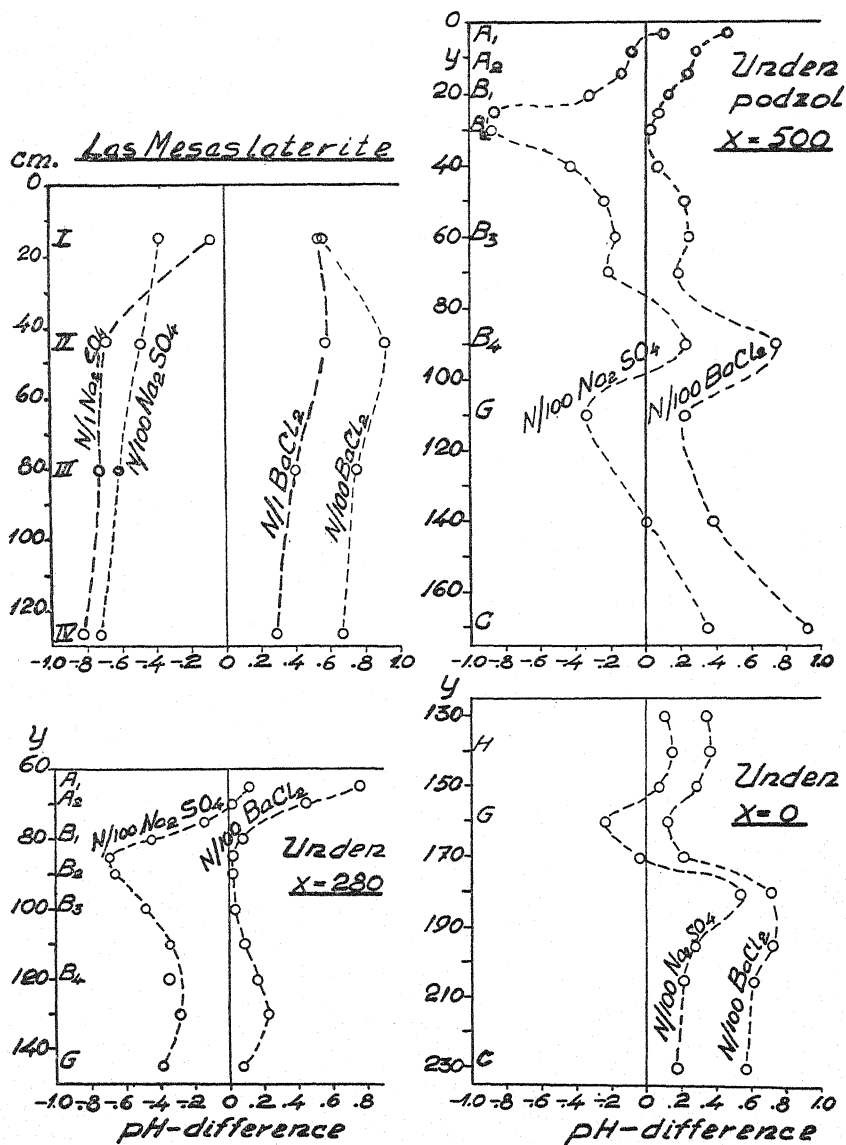


FIG. 101. DIFFERENCES BETWEEN THE pH IN WATER AND IN THE SALT SOLUTIONS OF THE LAS MESAS LATERITE AND OF THREE OF THE UNDEN PODZOL PROFILES

Negative values = exchange alkalinity; positive values = exchange acidity

tivity is greatest in sample II, whereas the acidoid activity is greatest in sample I, which contains considerable humus acidoids.

The differences between the pH values in water and in the salt solutions are shown graphically in figure 101. We get here a different picture from that

of the podzol profiles. The characteristic maxima and minima in the podzols are absent in the laterite. The exchange alkalinity of the laterite in the sulfate solutions is lowest in the surface soil and attains a uniform high value in all the subsoil samples. The exchange acidity in BaCl_2 ought to be greater in sample I than in the three subsoil samples. That this is not the case must be ascribed to the SO_4 ions which, in spite of a prolonged electrodialysis (1 week), still remained in samples II, III, and IV, as was shown by extracting the soil with a hot ammoniacal solution of NH_4Cl . Since these subsoil samples yielded a still greater exchange acidity before being electrodialyzed, we conclude that the exchange acidities of these samples are still "abnormally" high, whereas the exchange acidity of sample I more nearly expresses the true amphoteric nature of the soil complex.

Another significant thing in figure 101 (cf. Las Mesas) is the fact that the exchange alkalinity is greater in N than in $0.01 N \text{Na}_2\text{SO}_4$ and that the exchange acidity is greater in $0.01 N$ than in $N \text{BaCl}_2$ in samples II, III, and IV.

TABLE 156

The loss on ignition and the pH of the electrodialyzed Las Mesas laterite in water and in salt solutions

SAMPLE	DEPTH	LOSS ON IGNITION	pH IN				
			Water	$0.01N$ Na_2SO_4	$N \text{Na}_2\text{SO}_4$	$0.01N$ BaCl_2	$N \text{BaCl}_2$
	<i>inches</i>	<i>per cent</i>					
I	0-12	18.53	4.25	4.63	4.33	3.70	3.72
II	12-23	16.52	6.11	6.60	6.79	5.20	5.54
III	26-38	15.89	5.60	6.21	6.33	4.85	5.20
IV	43-58	16.75	5.79	6.51	6.61	5.12	5.50

This tells us that the basoid groups are so strong in these samples that the effect of the SO_4 ions *remains* stronger than that of the Na ions even in high concentrations, and that the effect of the Cl ions *becomes*, in high concentrations, greater than that of the Ba ions. In sample I the acidoid group is sufficiently strong to make the effect of the Na ions outweigh that of the SO_4 ions in high concentrations. The same tendency is shown with respect to the Ba and Cl ions.

OTHER APPLICATIONS

It has been pointed out that, theoretically, it would be possible to remove virtually all the anions and cations from a solution by passing it through alternate layers, or a coarse mixture, of acidoids and basoids. In order to find out to what extent this is possible in the case of soil acidoids and basoids, we performed the following experiment:

Twenty grams of electrodialyzed raw humus and fifty grams of Las Mesas II were separately moistened (to prevent intimate mixing and mutual interaction) with $0.01 N \text{CaSO}_4$ and then lightly mixed and placed in a tall glass funnel. The mixture was then leached with the CaSO_4 solution. The first,

second, third, and fourth 50-cc. portions of the filtrate collected showed no trace of Ca or SO_4 ions. A special study of this problem is planned.

The Donnan distribution of ions in the soil has many other important consequences. Consider, for example, the effect of a concentration and a dilution of the soil solution on the mobility of the monovalent and divalent ions. A dilution favors the entrance (in the soil complex and in the roots of plants) of the divalent and the outgo (in the soil solution) of the monovalent ions. A concentration reverses the process. One might say that acidoids and basoids "inhale" divalent and "exhale" monovalent cations and anions, respectively, upon wetting and "inhale" monovalent and "exhale" divalent cations and anions upon drying.

The "*dilution effect*" on alkali soils observed by Eaton and Sokoloff (1), and by Kelley (2) is obviously an expression of such an ionic "respiration" or a readjustment of an equilibrium, between ions of different valence, which has been disturbed through a change in concentration in the outside solution. Eaton and Sokoloff found that

The amount of sodium, both relative and absolute, in the aqueous phase tends to increase as the water-soil ratio is increased, whereas the relative concentration of calcium (and magnesium in some cases) tends to decrease. The absolute amount of calcium in solution may remain unchanged or tend to increase with dilution when soils contain an excess of sparingly soluble salts such as CaCO_3 and CaSO_4 .

This "*CaCO₃ effect*," which tends to obscure the valence effect, is very significant in the reclamation of calcareous alkali soils. In a noncalcareous alkali soil the dilution (or leaching) effect would not be very great because the mobile Ca ions would soon be exhausted. In a calcareous soil the Ca reserve is great and, since "the energy of adsorption" of the Ca ions is greater than that of the Na ions (*in dilute solution*), the low solubility of CaCO_3 is here an obvious asset: it allows the soil complex to take a "deep and prolonged breath" of Ca ions when the soil is leached, even when the irrigation water contains a few milliequivalents of alkali salts per liter.

Since a leached soil means a dilute soil solution, we can readily understand why, in the humid soils, the divalent base-forming cations are present in the exchange complex in a greater proportion than in the original silicate rocks and in the soil solution.

As to the dilution effect on the anions, that will depend on the amphoteric nature of the soil and on the pH at which the analysis is made. High above the equi-ionic point the anions are negatively adsorbed [$y < x$ in equations (K) and (L)]. At lower pH, at which the basoids are functioning, the anions are positively adsorbed ($y + z > x$) and the dilution effect, with respect to the valence, will be the same as that for the cations at high pH, provided there is no hydrolysis and no solution effect (CaSO_4 etc.). The investigator must know the amphoteric character of his soil, its equi-ionic point, the pH at which he is working, the volume of the micellar solution, and many other things before he can, even qualitatively, interpret his results.

CONCLUSION

The dilution effect leads Kelley to the noteworthy conclusion that "there is no such thing as a definite or true soil solution." This statement emphasizes the dynamic character of the soil, which is subject to constant changes in response to its environment. The truth of the statement is best realized when we consider that hand in hand with the redistribution of the ions, resulting from changes in concentration, there are changes in potential difference, in the osmotic and electrical transfer of water (swelling and shrinking), in dispersion and Brownian mobility, and in many other properties. The amphoteric character of the complex and the conception of an amphoteric ion atmosphere make the picture more complicated. Clusters or "clouds" of ions of opposite sign of charge, Brownian bombardments, charges, and discharges must give rise to a turbulence comparable to the electrical storms in our terrestrial atmosphere.

There is perhaps nothing which better testifies to the inconstancy of soil factors than the pH and the capacity to bind base. Kelley might have included in his statement that *there is no such thing as a definite or true soil pH or any definite or true soil capacity to bind base (or acid)*. Apart from the changes in the activity coefficients with changes in the ionic strength of a solution, common to all electrolytes, we meet, in colloidal electrolytes, the peculiar conditions of an unequal distribution of ions. This distribution is affected by salts in such a way that the pH of a soil can be both higher and lower in solutions of different concentrations of the same salt than the pH of the soil in water. As to the capacity to bind base at a given pH, we know from our titration curves that this capacity will be different in different solutions. Above the point of exchange neutrality, of two concentrations the capacity will be greater in the stronger solution, whereas below this point it will be greater in the dilute solution.

Since the pH of a given soil at a given base status is a function of the concentration and composition of the soil solution and since the net capacity of the soil to bind base at a given pH is an expression of the power of the base-forming cations to displace the H ions of the acidoids, over and above the power of the acid anions to displace the OH ions of the basoids, and further, since the relative displacing power of ions of different valence changes with changes in concentration, we arrive at the conclusion that *the pH, the concentration and the composition of the soil solution, and the capacity of the soil to bind base at a given pH are interdependent variables*. This conclusion may be summed up in the following statement: *At each pF of the soil there is a different soil solution, a different pH, and a different capacity to bind base*. The soil problems are complicated, but it has been said that "*it is easier to find your way in a natural cave than in a man-made labyrinth*," and the soil is a natural body.

SUMMARY

The property of amphoteric soils simultaneously to exchange H and OH ions for the cations and anions of a neutral salt solution at, or near, the equi-

ionic point of the soil, and the valence effect in the mass law (as expressed by the Donnan distribution of ions between the soil complex and the soil solution) lead to some very significant, but heretofore neglected, consequences.

For the salts Na_2SO_4 and CaCl_2 the following expressions apply:
for acidoids:

$$\frac{(\text{H}^+)_i}{(\text{H}^+)_o} = \frac{(\text{Na}^+)_i}{(\text{Na}^+)_o} = \frac{\sqrt{(\text{Ca}^{++})_i}}{\sqrt{(\text{Ca}^{++})_o}}$$

and for basoids:

$$\frac{(\text{OH}^-)_i}{(\text{OH}^-)_o} = \frac{(\text{Cl}^-)_i}{(\text{Cl}^-)_o} = \frac{\sqrt{(\text{SO}_4^{--})_i}}{\sqrt{(\text{SO}_4^{--})_o}}$$

where the parenthesis represents activity and i and o signify that the ions are in the inside and the outside solutions respectively.

The fact that the divalent ions enter the equation in the form of the square root of their activity indicates that, in low concentrations (in the "outside" solution) of the salt and in the presence of equivalent proportions of free acidoids and basoids, the number of H ions displaced by the Ca ions will be greater than the number of OH ions displaced by the Cl ions, and the number of OH ions displaced by the SO_4 ions will be greater than the number of H ions displaced by the Na ions. In high concentrations the displacing power of the different ions will approach the same value. These conclusions are all drawn with the proviso that none of the ions enter into any specific reaction.

From this it follows that, under the aforementioned conditions, CaCl_2 (or a salt of the type $\text{M}^{++}\text{S}^{--}_2$) must yield a maximum in exchange acidity in dilute solutions and Na_2SO_4 (or a salt of the type $\text{M}^{+}_2\text{S}^{-}$) must yield a maximum in exchange alkalinity in dilute solutions.

A theoretical study has been made of the various factors which determine the position and magnitude of these maxima, such as the acidoid/basoid ratio, the concentration of colloid, and the concentration of the ions in the micellar solution.

By means of titrations and pH determinations of different soils in various concentrations of salts, it has been possible to prove the application of the theory.

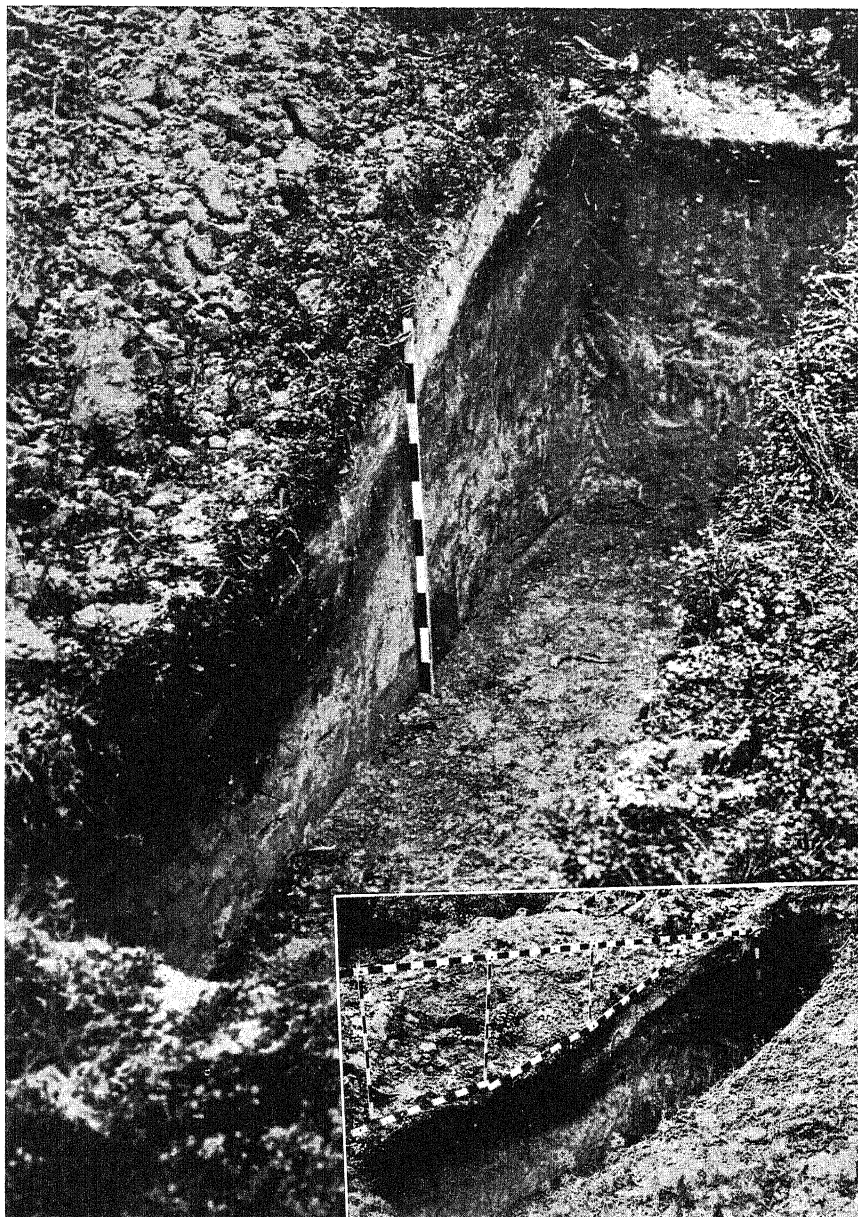
REFERENCES

- (1) EATON, F. M., AND SOKOLOFF, V. P. 1935 Adsorbed sodium in soils as affected by the soil-water ratio. *Soil Sci.* 40: 237-247.
- (2) KELLEY, W. P. 1939 Effect of dilution on the water-soluble and exchangeable bases of alkali soils and its bearing on the salt tolerance of plants. *Soil Sci.* 47: 367-375.
- (3) MATTON, S., AND GUSTAFSSON, Y. 1935 The chemical characteristics of soil profiles. II. The mutual interactions of podzolic materials. *Ann. Agr. Col. Sweden* 2: 1-30.
- (4) MATTON, S., AND LÖNNEMARK, H. 1939 The pedography of hydrolytic podzol series: I. Loss on ignition, pH and amphoteric reactions. *Ann. Agr. Col. Sweden* 7: 185-227.

PLATE 4

THE UNDEN HYDROLOGIC PODZOL SERIES

SANTE MATTSON AND LAMBERT WIKLANDER



BASE EXCHANGE IN SOILS: II. EXCHANGE ACIDITY

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When a neutral salt is shaken with an acid soil a portion of the cation of the salt is taken up and an equivalent amount of free acid is produced. This is known as the exchange acidity of the soil and is sometimes referred to as the potential acidity or exchangeable hydrogen. When the salt of a weak acid and a strong base, like sodium acetate, is used, the amount of titrable acidity produced is much larger than in the case of a neutral salt and is known as hydrolytic acidity.

It is well known that the distribution of a base between two acids is governed by the relative strengths of the acids competing for the base, the stronger acid combining with the larger proportion of the base. In other words, if the relative amounts of the individual salts formed could be estimated, the relative strengths of the acids in a mixture would be known. Similarly if a salt is added to a soil acidoid, the amount of the basic portion of the salt going over to the soil acidoid will be governed by the relative strength of the acid radical of the salt as compared to the soil acidoid. For instance, a larger proportion of the base would be taken up by the soil from acetates than from chlorides.

It is the object of this paper to present evidence that the whole phenomenon of exchange acidity is due to the distribution of a base between two acids and is entirely governed by the relative strengths of the acid and the soil acidoid, and that any distinction between exchange acidity and hydrolytic acidity is arbitrary and misleading.

One of the most accurate methods of following the distribution of a base between two acids is the measurement of pH values. When a mixture of equivalent amounts of two acids of unequal strength is titrated with a base, the titration curve is identical with that of the stronger acid at the start, and if the dissociation constants of the two acids are far removed, virtually all of the stronger acid is neutralized before the weaker acid is affected. From the nature of these titration curves, it is possible to judge the relative strengths of the acids. On the other hand, the change in the pH value of a H-soil, on the addition of an equivalent amount of a salt of an acid, will be a measure of the strength of the acid.

The equivalent point of a H-soil can be determined from its titration curve and is referred to as its $T/2$ value.¹ Four soils of widely differing character-

¹ Puri, A. N., and Asghar, A. G. 1938 Titration curves and dissociation constants of soil acidoids. *Soil Sci.* 45: 359-367.

istics were used in the first instance. The soils were freed from bases by treatment with 0.05 *N* HCl; equivalent amounts of K salts of various acids were added to these H-soils, and pH values were determined with the glass

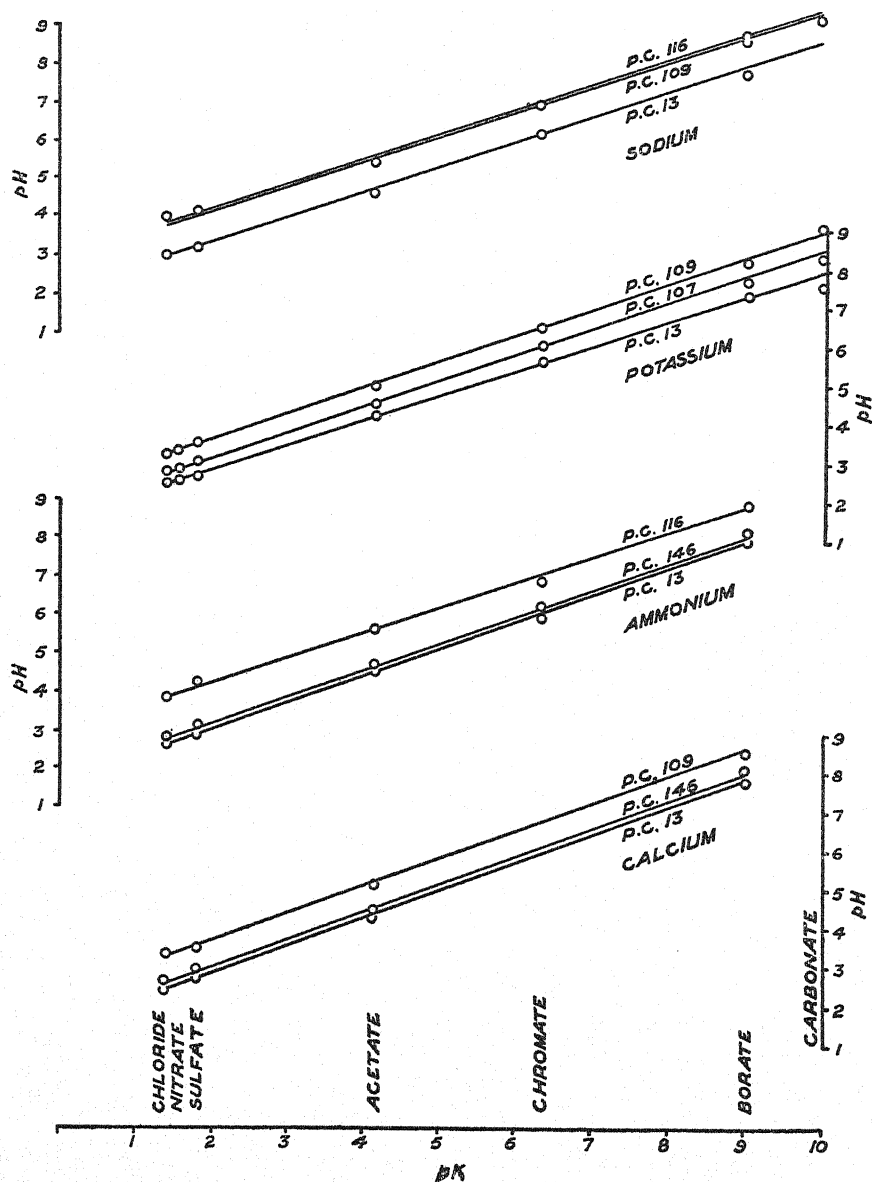


FIG. 1. RELATION BETWEEN pH AND pK VALUES OF VARIOUS SALTS ADDED TO H-SOILS

electrode. When pH values of the resulting mixtures are plotted against the logarithms of their dissociation constants, i.e., the pK values, a series of parallel lines for various soils are obtained (fig. 1). Thus if the pH value

of a H-soil in KCl solution is determined, its pH value in any other solution of the K-salt of the acid of which the pK value is known is given by the formula:

$$\text{pH} = .57 (\text{pK} - 1.4) + \text{pH KCl} \quad (A)$$

It is, of course, understood that the various salts and the H-soil are used in equivalent amounts.

The pK values for the various acids used are as follows:

Acetic acid	= 4.2
Chromic acid	= 6.4
Boric acid	= 9.2
Carbonic acid	= 10.2
Nitric acid	= 1.5
Sulfuric acid	= 1.8
Hydrochloric acid	= 1.4

These acids in the concentrations developed in soils have no action on the clay complex. Formula (A) is applicable to K salts. For Na, NH_4 , and Ca salts, the following formulas were worked out from the straight line relationships:

$$\text{pH} = .58 (\text{pK} - 1.4) + \text{pH NaCl} \quad (B)$$

$$\text{pH} = .58 (\text{pK} - 1.4) + \text{pH NH}_4\text{Cl} \quad (C)$$

$$\text{pH} = .69 (\text{pK} - 1.4) + \text{pH CaCl}_2 \quad (D)$$

The calculated pH values for various soils in different salt solutions in comparison with those determined experimentally are given in tables 1 and 2. In table 2 a larger number of soils are compared for acetates only. In every case 5 gm. of the soil was shaken for 48 hours with an equivalent amount of the various salts in 100 cc. of solution, and pH values were determined with the glass electrode. The calculated and found pH values show a good agreement for all types of soils studied and leave no doubt as to the nature of the exchange reactions involving the displacement of H ions.

The relation established in the foregoing is true only when equivalent amounts are used. It would be interesting to know how increasing amounts of salts will affect the distribution of the base. From the titration curve of an acid, we can imagine any point on the curve as representing an acid which is weaker than the original acid. If we are dealing in terms of H ions only, a series of acids of decreasing activity can be obtained by partially neutralizing any acid with increasing amounts of a base. For instance, the pK values of one-fourth, one-half, and three-fourths neutralized acetic acid are 4.7, 4.95, and 5.40 respectively.

We can regard an equivalent amount of any of these partially neutralized acids in the usual manner as containing 1 gram molecule of replaceable H. When they are neutralized the net result will be salts of increasing concentration. Alternately, we can regard increasing concentrations of a salt as the equivalent of a base neutralized with acids of decreasing activity. This con-

TABLE 1
Calculated and found pH values of H-soils in different salt solutions

SALTS	P.C. 13		P.C. 146		P.C. 109		P.C. 107		P.C. 116		P.C. 61		P.C. 145		P.C. 147		P.C. 123		
	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	Found	Calcu- lated	
K	chloride.....	2.7	2.8	3.4	3.0	3.4	2.8	2.8	3.5
	nitrate.....	2.7	2.75	2.8	2.85	3.3	3.45	3.0	3.35	3.5	3.45	2.86	2.85	2.82	2.85	3.5	3.55
	acetate.....	4.5	4.35	4.5	4.4	5.0	5.0	4.6	4.6	4.7	5.0	4.4	4.4	4.4	4.4	4.55	5.1
	borate.....	7.2	7.1	7.5	7.2	8.26	7.8	7.5	7.4	7.6	7.8	7.2	7.2	7.3	7.2	7.2	7.9
	sulfate.....	2.8	2.9	2.9	3.0	3.7	3.6	3.3	3.2	3.9	3.6	3.1	3.0	3.0	3.0	3.8	3.7
	chromate.....	5.7	5.6	5.9	5.7	6.6	6.3	6.1	5.9	6.4	6.3	6.1	5.7	6.1	5.7	6.1	6.4
Na	carbonate.....	7.3	7.7	7.5	7.8	9.3	8.4	8.3	8.0	8.6	8.4	N.D.	7.8	N.D.	7.8	7.3	8.5
	chloride.....	3.0	2.9	4.0	4.0	4.2	4.2	
	sulfate.....	3.0	3.2	3.0	3.1	4.1	4.2	4.2	4.2	4.1	4.3	4.3	4.4	
	acetate.....	4.5	4.6	4.5	4.5	5.2	5.6	5.3	5.6	5.2	5.8	5.1	5.8	
	chromate.....	6.0	5.9	6.1	5.8	6.6	6.9	6.7	6.9	6.4	7.1	6.3	7.1	
	borate.....	7.4	7.5	7.5	7.4	8.3	8.5	8.4	8.5	8.1	8.7	7.8	8.7	
Ammo- nium	carbonate.....	8.9	8.1	9.2	8.0	9.5	9.1	9.9	9.1	9.5	9.3	9.3	9.3	
	chloride.....	2.6	2.7	3.8	
	sulfate.....	2.8	2.83	3.0	2.9	4.3	4.03	
	acetate.....	4.4	4.2	4.4	4.3	5.5	5.42	
	chromate.....	5.8	5.5	5.9	5.6	6.6	6.7	
	carbonate.....	7.75	7.7	7.9	7.8	8.5	8.9	
Ca	chloride.....	2.5	2.65	3.45	
	sulfate.....	2.8	2.78	2.95	2.93	3.50	3.73	
	acetate.....	4.4	4.43	4.4	4.5	5.3	5.38	
	borate.....	7.9	7.88	8.1	8.03	8.7	8.8	

N.D. = not determined.

ception may help us in visualizing the mechanism of pH changes of a H-soil with increasing amounts of salt. If we plot the pH values with different concentrations of a salt against the calculated pK values, the results fall on straight lines characteristic of different acids which branch off from the curve given in figure 1. This relation is shown in figure 2 on a magnified scale. HCl, being completely ionized, the same pH is obtained at all concentrations; consequently, our starting point with KCl may be taken as fixed, and the formula developed can be used for finding the pH value of a H-soil in any solution at any concentration, provided we know the pH on the addition of an equivalent amount of the salt from formulas (A) to (D). The equation giving the relation between pH and pK values at different concentrations is derived as follows:

TABLE 2
Calculated and found pH values of H-soils in K-acetate

SOILS	pH WITH KCl	pH WITH K ACETATE		T/2 VALUES <i>m.e./100 gm.</i>
		Found	Calculated	
P.C. 2	3.0	4.6	4.6	55.0
6	3.4	4.8	5.0	12.0
7	4.4	5.5	6.0	8.0
15	3.8	5.1	5.4	5.0
32	3.3	4.9	4.9	58.0
68	4.1	5.4	5.7	18.0
70	3.6	5.4	5.2	48.0
73	3.6	4.9	5.2	28.0
110	4.1	5.4	5.7	10.0
114	3.6	5.5	5.2	40.0
115	3.9	5.6	5.5	30.0
116	3.9	5.3	5.5	19.0
122	3.8	5.3	5.4	8.0
124	3.5	5.1	5.1	15.0
132	3.2	5.0	4.8	18.0
138	3.8	5.0	5.4	10.0
152	3.6	5.2	5.2	36.0
153	3.85	5.5	5.45	44.0

Equations (A) to (D) can be written in the general form:

$$\text{pH} = m (\text{pK} - \text{pK}_0) + \text{pH}_0 \quad (E)$$

in which pH_0 is the pH value when the soil is treated with KCl at equivalent concentration; pK_0 is the dissociation constant of HCl (1.4); pK is the dissociation constant of the acid of which the salt is used; and m is the slope of the line.

For the equation of any straight line that branches off from this line at any point (i.e., the line showing the relation between concentration and pH), the equation will be:

$$\text{pH}_1 = m_1 (\text{pK}_1 - \text{pK}) + \text{pH} \quad (F)$$

in which pH is the value when the concentration of the salt is equivalent to that of soil and which is given by formula (E); pK_1 is the dissociation constant of the acid in accordance with the assumption made above; and m_1 is the slope of the line showing the effect of concentration on pH.

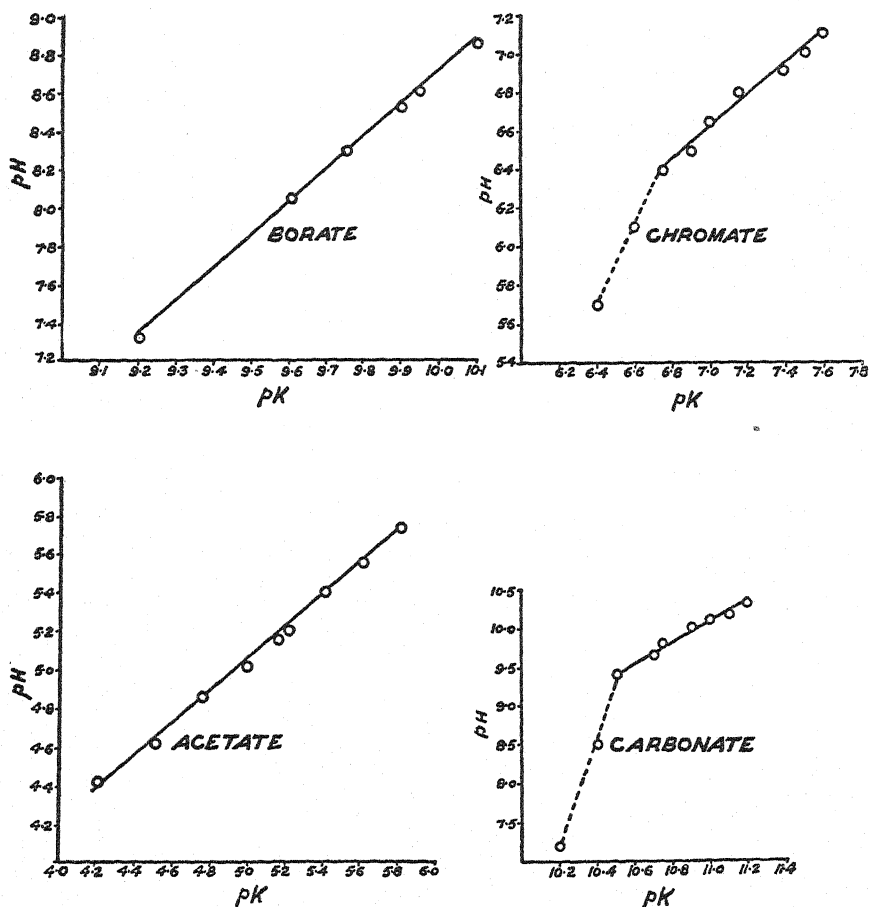


FIG. 2. EFFECT OF CONCENTRATION OF THE SALT ON ITS pH VALUE WITH H-SOILS

Substituting numerical values in equation (F) in the case of soil P.C. 13 A.T. we have for acetates:

$$pH_1 = .835 (pK_1 - 4.2) + 4.3$$

and for borates:

$$pH_1 = 1.72 (pK_1 - 9.2) + 7.3$$

The values of pK_1 are, of course, calculated for every concentration in accordance with the assumption that a higher concentration of salt may be

taken as the salt of a weaker acid. The following relation is used for finding the pK_1 value; if C_1 is the concentration of the salt, and C_2 is the concentration equivalent to the soil acidoid, then the pK_1 value of the partially neutralized acid equivalent to the soil acidoid is given by the pH of the acid neutralized to $100 \left(1 - \frac{C_2}{2C_1}\right)$ per cent. This value is interpolated from the titration curve of the acid. An example will make this point clear. Suppose we want to find the pH value of a solution containing 10 m.e. of K-acetate when shaken with a H-soil containing 2 m.e. of acidoid. The pK_1 value of the partially neutralized acid will be equal to pH when $100 \left(1 - \frac{2}{2 \times 10}\right) = 90$ per cent

TABLE 3
Calculated and found pH values of K-acetate and K-borate at various concentrations

CONCENTRATION	pK	pH	
		Found	Calculated
Acetate			
N/40	4.2	4.3	4.3
N/26.6	4.75	4.85	4.75
N/20	5.0	4.95	4.96
N/16	5.15	5.15	5.1
N/13.3	5.2	5.2	5.12
N/10	5.4	5.4	5.30
N/6.6	5.6	5.55	5.46
N/5	5.8	5.72	5.63
Borate			
N/4	9.2	7.3	7.3
N/26.6	9.6	8.05	7.98
N/20	9.75	8.25	8.24
N/16	9.9	8.5	8.5
N/13.3	9.95	8.6	8.59
N/10	10.05	8.75	8.76

of acetic acid is neutralized with KOH. From the titration curve of acetic acid with KOH this is found to be 5.6.

Substituting this value in formula (F) we have:

$$pH_1 = .835 (5.6 - 4.2) + 4.3 = 5.46.$$

The calculated and found values for the various concentrations are given in table 3.

When dealing with salts of dibasic acids like chromic and carbonic, a straight line relation is not obtained at low concentrations. The reason is obvious. These acids have a point of inflection at the end of the first half of the neutralization when the conditions are highly unbuffered, and therefore a slight

change in the degree of neutralization can produce a large difference in the pH value. The measurement of pH values is not reliable in this region, and it is not certain whether one is dealing with the first or the second dissociation constant unless one works with concentrations so high that they can only fall in the second half of the titration curve. The effect of concentration of the salt on the pH value of the soil acidoid in the case of chromates and carbonates shown in figure 2 has not been included in the mathematical part of this study. Sulfates, on the other hand, fall in line with salts of monovalent acids, because there is no point of inflection at the end of the first half of the neutralization. It is to be noted that salts of dibasic acids are used in molecular concentrations, and therefore an equivalent of twice the amount of acidoid is employed to ensure that one is dealing with the dissociation constant of the mono acid salt. Phosphates have been excluded from this study because of the possibility of side reactions. The data presented, however, leave no doubt as to the fundamental cause of exchange acidity, namely, the distribution of a base between two acids of unequal strength.

SUMMARY

The phenomenon of exchange acidity in soils has been studied. The results show that the production of free acid on the addition of a salt to an acid soil is governed by the law of distribution of a base between two acids of unequal strength.

THE OXIDATION OF MANGANOUS COMPOUNDS BY MICROORGANISMS IN THE SOIL

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There is considerable evidence to indicate that the oxidation of manganous compounds to manganic oxide may be used as a source of energy by microorganisms. Söhngen (7) and Beijerinck (1) studied certain aspects of this oxidation. Gerretsen (2, 3) pursued the subject in the course of his work on manganese deficiency of oats, using the following method of investigation: The soil to be studied (30 gm.) is mixed with water (25 ml.) and 100 ml. of 2 per cent agar at 40°, in a petri dish 11 cm. in diameter. When the mass is solid, a hole 2.5 cm. in diameter is cut from the center, and agar containing 1 per cent MnSO_4 , usually mixed with sand, is poured into the gap. The dish is then incubated at 25°, and the development of brown deposits of manganic oxide is noted during the following days.

Gerretsen adjusted the pH of his cultures by the addition of HCl or NaOH, and found that after 2 weeks' incubation manganic oxide was developed only between the limits of pH 6.3 and 7.8, with a maximum at 7.0 and only slight development at pH 6.4 and 7.3. This alkaline limit seemed to us so unlikely to be a general property of soils that we applied the same technic to a number of soils and found that the limits are, in general, much wider than those just quoted. Some of the observations made in the course of this work seem of sufficient interest to be placed on record.

The methods used were those of Gerretsen as quoted, except for minor details; incubation was at 27° instead of 25, sand was omitted from the central plug of MnSO_4 , and the amounts of soil and agar were reduced usually to one-fourth of those quoted. Sulfuric acid or sodium hydroxide was added to each of the soils in such amounts that the final agar plaques covered the range of pH from 5.0 to 9.0. Most of the pH values quoted were determined either with the quinhydrone electrode (where reactive manganic oxide was not present) or colorimetrically. Some of the extreme values were checked with the glass electrode.

One difficulty which immediately arises is the fact that, during incubation, the pH of the soil-agar plaques falls from 0.3 to 0.9 of a unit, on account of the formation of acids from the more available carbohydrates of the medium. The tables therefore indicate whether the pH value refers to the original plaque (actually to the central plug which was removed to make room for

the MnSO_4 -agar) or to the plaque at the end of the experiment. Possibly the mean of these figures should be used in discussing the results.

Gerretsen established the biological nature of the reaction by showing that plaques to which chloroform had been added did not develop any brown spots of manganic oxide. We repeated this work on several plaques, using either chloroform or germisan as a disinfectant, and confirmed his results.

The soils used, and referred to by their place names, were as follows:

Chocolate sandy loam from *Mt. Gambier*, South Australia. Highly immature, derived from volcanic ash, and calcareous; pH 7.4.

Black clay from *Penola*, South Australia. Reclaimed swamp, self-mulching when dry. pH 6.8.

Light gray sandy loam from *Timboon*, Victoria. Poor podzolized soil, recently limed; pH 7.5.

Gray sandy loam from the grounds of *Melbourne University*. Limed to pH 7.3, 15 years ago.

Ferruginous gravelly sand from Western Australian wheat belt ("W.A. Gravel"); pH 6.9.

Highly acidic sand from *Cranbourne*, Victoria. Recently limed.

Gray calcareous clay, from *Dooen*, Victoria. Self-mulching; pH 7.3.

Dark gray calcareous clay from *Werribee*, Victoria. Derived from Pleistocene basalt; pH 7.8.

References to several of these soils have been made in another report (4).

The first six of these soils are associated with manganese-deficiency disease, and the last two are not. The Werribee soil contains so much active manganic oxide that its pH cannot be measured by the quinhydrone electrode. All samples were taken directly from the field or from pots which were exposed to the weather; consequently, there was no complication through possible changes in the microbial flora by air drying.

RESULTS

The samples studied in this work show a remarkable range in behavior. The technic is so simple and the results so varied and so striking that the test may be recommended for purposes of demonstration. Plate 1 shows some of the patterns of the precipitated oxide. The staining power of the 2 mgm. of manganese used is remarkable. Each soil gave a characteristic pattern which was reproducible within a certain range of pH. Some soils developed a uniform brown zone (pl. 1, fig. 1), others developed a ring of brown spots up to about 0.5 mm. in diameter (figs. 5 and 6), others slowly formed large spots, up to 5 mm. diameter, without any ordered arrangement (fig. 2). This random distribution of the slowly formed spots is due to the fact that manganese diffused uniformly over the plaque before precipitation began. Many plaques, especially in the most acidic and the most alkaline ranges, developed spots on the reverse (lower side) and not on the surface (upper side); in view of the aerobic nature of the reaction, this fact is curious, and is presumably connected with the growth of fungi or other bacteria on the surface but not on the reverse. The distance of the brown rings from the central plug, their

width, and their extension inward or outward during the second and third weeks of incubation were also very different with different soil types. The original formation of a ring at a distance as great as 1 cm. from the central plug is due to toxic concentrations of manganous ion closer to the center. Thus, sandy soils develop the ring farther from the center than clayey soils. If lower concentrations of manganous sulfate in the central plug are used, the precipitation takes place without any such gap. In only two out of a hundred plaques did brown spots fade after once forming.

TABLE 1
Alkaline limits of biological oxidation of manganous ion for various soils

SOIL	ALKALINE LIMIT		REMARKS
	pH*	Time to first brown spot	
Penola	8.5 F	9	Brown spots on reverse, zone 1 cm. wide. $MnCO_3$ on surface, 5 mm. wide, 1 day after start; with brown zone outside, not spotty, after a week
	8.9 F	20	
University	8.3 F	3	Ring of brown spots on reverse, fading and disappearing after a week. Brown ring, not spotty, 5 mm. wide, outside $MnCO_3$ on surface
	8.8 O	7	
W. A. Gravel	8.5 F	60	Well marked only after 21 days (fig. 3)
	8.9 O		
Werribee	8.5 F	20	Not well marked on surface, a few brown spots on reverse
	8.6 F		
	8.4 F		
			Brown spots on reverse, not well marked.
			Brown spots on reverse, well marked at 14 days.
			Brown ring on surface not spotty, showing clearly outside $MnCO_3$. (This brown ring did not appear on a parallel plaque containing 0.8 per cent germisan in the total medium)

* O = original; F = final.

We did not attempt to separate or identify the microorganisms responsible for these changes. Apparently Beijerinck (1) is the only worker who has attempted this task.

The precipitated manganic oxide is stained blue by benzidine salts; its composition presumably lies somewhere between the limits of Mn_2O_3 and MnO_2 . In some plaques (e.g., pl. 1, fig. 5) the outermost spots were rust-colored and the inner spots were darker brown.

The upper and lower limits of pH at which biological oxidation occurs for several soil types are collected in tables 1 and 2.

At pH values above about 8.0 a white precipitate of MnCO_3 , 2 to 5 mm. wide, formed on the surface, immediately outside the central plug. A brown ring developed outside this on several plaques on both surface and reverse, due to biological oxidation, as was proved by its prevention with germisan. At still more alkaline levels biological oxidation did not take place, and a slow nonbiological oxidation was shown by the gradual darkening of the precipitated MnCO_3 and by a dark cloudiness over the inner plug of MnSO_4 -agar.

The biological oxidation of manganese is not so greatly affected by increas-

TABLE 2
*Acid limits of biological oxidation of manganous ion for various soils**

SOIL	ACID LIMIT		REMARKS
	pH†	Time to first brown spot days	
Gambier	5.6 O	7-28	Enormous development after 2 months, on reverse
Penola	5.9 O	7-28	Well-marked spots on reverse only, in ring 12 mm. from plug
	5.6 O	6	
	4.8 F		Two months after incubation. Spots also on surface
University	6.2 O	6	Well marked, surface and reverse. Ring 2 cm. from plug, gradually extending to outer edge. Much mold on surface
	5.3 F		
	5.8 O	7-28	Best marked on reverse: relatively few spots, very large (5 mm.), uniformly scattered over whole of plaque (fig. 2).
Werribee	6.0 O	3	Very well marked (fig. 4).
	5.7 F		
	5.1 F	5	Few spots on reverse, gradually increasing in number

* These results were all obtained with artificially acidified soils. Similar results have been obtained with naturally acidic soils.

† O = original; F = final.

ing acidity as might be expected. According to Wolzogen-Kühr (8) bacteria can oxidize dissolved manganese at pH values between 4 and 5. Our own experiments indicate that soil organisms can oxidize manganese, provided the initial pH value of the soil-agar plaque is 5.5 or more. One acidified plaque developed brown spots in a broken ring. This suggested that the intervening spaces containing no spots were too acidic for the oxidation of manganous ions (which incidentally involves the consumption of hydroxide ions). Tests with the glass electrode showed that the pH was 5.1 where the brown spots had developed and 4.8 where they had not.

APPLICATION TO REACTIONS IN THE FIELD

One must not assume too readily that these changes in agar plaques represent the reactions which take place in the field. The plaques contain 1 part of agar to 15 of soil; and this agar provides an unusual environment for the microorganisms. A batch of plaques prepared with kaolin as the diluent instead of agar gave few positive results. Kaolin plaques present obvious difficulties as to aeration.

Rate of oxidation

The brown spots develop most rapidly in plaques of pH between 6.0 and 7.5. This rate of development is of considerable interest. The concentration of bivalent manganese in the soil solution, or as a readily exchangeable ion attached to the colloidal material, depends on the relative rates of its liberation and its removal. The bivalent ion may be liberated by the weathering of minerals, the reduction of reactive manganic oxides by organic matter, or the decomposition of plant residues and dead microorganisms, which contain manganese in organic combination; it may be removed by oxidation, as described here, or by absorption into living microorganisms or roots. The equilibrium concentration of manganous ions in solution, other things being equal, will be decreased as the rate of bacterial oxidation is increased.

Manganese deficiency disease of oats (gray speck)

Gerretsen (2, 3) has suggested that the maximum incidence of manganese deficiency disease on soils between the pH values of 6.5 and 7.5 is connected with the ease of microbial oxidation of bivalent manganese within this range. Our results appear to give some support to his claim that these two phenomena are correlated, although we consider that his upper pH limit of 7.3 to 7.5 for microbial oxidation is too low; incidentally, the most important "deficient" soil type in Australia, the calcareous soil of Corny Point, South Australia, has a pH of 8.0. Bacterial oxidation, however, is only one aspect of this problem, and mere rapidity of oxidation of manganous ions is certainly not capable of making a soil "deficient." Some of the densest and most rapidly formed rings of manganic oxide appeared on soils which are known to be free from any deficiency disease; whereas the soil of Timboon, which was made deficient by liming to pH 7.5, gave the poorest development of manganic oxide among all the soils tested. Further, some healthy alkaline soils have been shown to contain less exchangeable manganese than do some "deficient" soils (4, p. 241).

The absence of microbial formation of manganic oxide on very alkaline soil-agar plaques calls for some comment. The limiting factor here may be the removal of bivalent manganese from solution as carbonate etc. Manganese deficiency disease has rarely been reported from soils of pH value above 8.0, and in fact the disease has been alleviated by adding calcium

hydroxide to the soil (5, 6). The senior author has found¹ that the growth of oats on "deficient" soil is greatly improved by raising the pH above 8.5 with caustic soda. Though this improvement may be due solely to a difference in the metabolism of plants growing on highly alkaline soils, it is also conceivable that roots can utilize manganous carbonate more readily than manganic oxide, and one would expect manganous carbonate to be present in greatest amounts in those environments in which it is least soluble and therefore most stable—that is, at high pH values. Beijerinck (1) used manganous carbonate as the source of energy when isolating bacteria and molds which would oxidize manganous ions. In this case, however, the environment was on the acid side, and therefore it does not concern this last argument.

SUMMARY

The microbial oxidation of manganous ions to manganic oxide was studied in soil-agar plaques, which were adjusted to various pH values. Brown spots due to microbial oxidation were observed on plaques of which the final pH ranged from 4.8 to 8.9.

REFERENCES

- (1) BEIJERINCK, M. V. 1914 Oxidation of manganous carbonate by microorganisms. *Verslag Akad. Wetensch.* 22: 415-420.
- (2) GERRETSEN, F. C. 1936 Een Onderzoek naar de Oorzaken der Veenkoloniale Haverziekte. *Verslag. Landbouwk. Onderzoek. Rijkslandbouwproefsta.* No. 42 A: 57-60.
- (3) GERRETSEN, F. C. 1937 Manganese deficiency of oats and its relation to soil bacteria. *Ann. Bot. (n.s.)* 1: 207-230.
- (4) LEEPER, G. W. 1935 Manganese deficiency disease of cereals; plot experiments and a new hypothesis. *Proc. Roy. Soc. Victoria (n.s.)* 47: 225-261.
- (5) MASCHHAUPT, J. G. 1934 Das Rätsel der Dörrfleckenkrankheit. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* (B) 13: 313-320.
- (6) POPP, M., CONTZEN, J., AND GERICKE, S. 1934 Das Rätsel der Dörrfleckenkrankheit. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* (B) 13: 66-73.
- (7) SÖHNGEN, N. L. 1914 Umwandlungen von Manganverbindungen unter Einfluss mikrobiologischer Prozesse. *Centbl. Bakt. (II)* 40: 545-554.
- (8) WOLZOGEN-KÜHR, C. A. H. VON. 1926 Het Mangan in het Amsterdamsche Duinwaterleidingbedrijf. *Water en Gas* 1926: 39-43. (Abs. in *Water and Water Engin.* 28: 216.)

¹ Unpublished data.

PLATE 1

SOIL-AGAR PLAQUES AFTER INCUBATION WITH $MnSO_4$ -AGAR IN CENTRAL PLUG

- FIG. 1. University (surface). Initial pH 7.3, final pH 6.7.
 FIG. 2. University (reverse). Initial pH 5.8.
 FIG. 3. University (surface). Initial pH 8.8, final pH 8.5.
 FIG. 4. University (reverse). Initial pH 6.0, final pH 5.7.
 FIG. 5. Doon (surface). Final pH 6.6.
 FIG. 6. Cranbourne (reverse). Final pH 6.6.
 (Diameter of each plaque 11 cm., depth 4 mm.)

FIG. 1

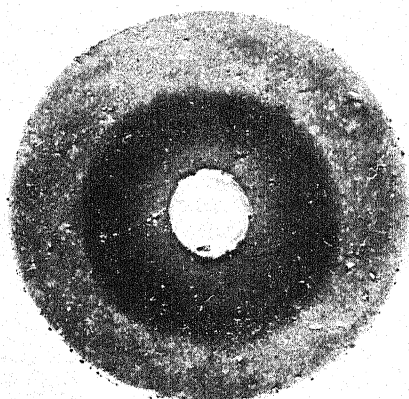


FIG. 2

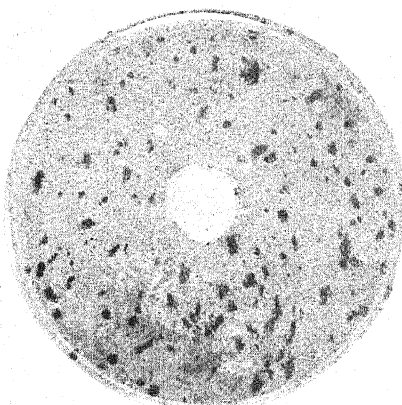


FIG. 3

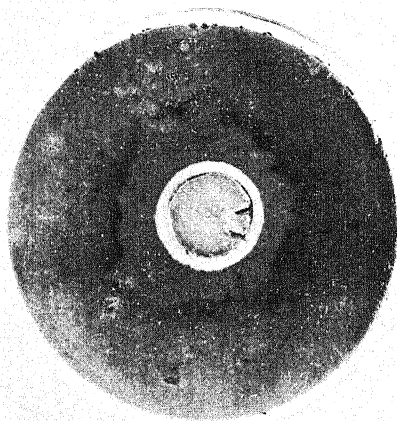


FIG. 4

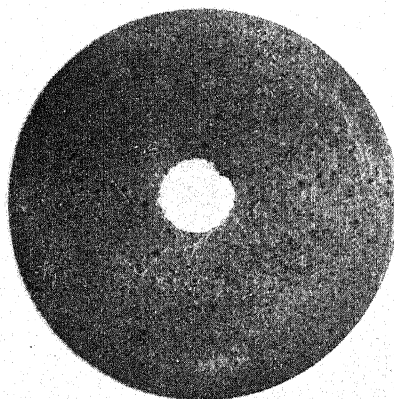


FIG. 5

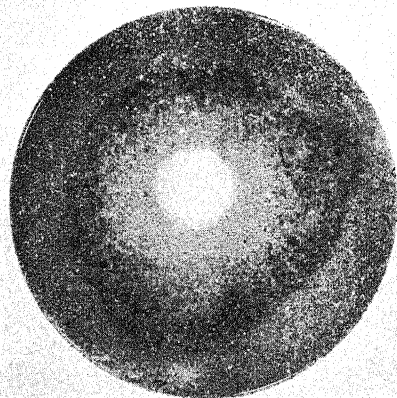
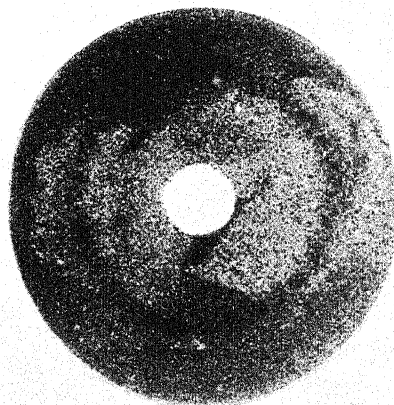


FIG. 6



THE INFLUENCE OF ENVIRONMENTAL FACTORS UPON THE DEVELOPMENT OF ALGAE AND OTHER MICRO-ORGANISMS IN SOIL¹

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It has been definitely established that algae form one of the components of the microbial population of the soil, but comparatively little is known concerning the importance of algae in soil processes. Certain species are known to be able to fix atmospheric nitrogen (1, 5, 6). These algae may, therefore, be considered as adding to the supply of nitrogen in the soil. Living and dead algae undoubtedly supply to the soil available organic matter which may be utilized by other microorganisms. It has been calculated that the total average bulk of the algae in the soil may in some cases be as much as three times that of the bacteria (4). The importance of algae in supplying oxygen to developing rice plants has also received some attention (10); this capacity to modify the composition of the soil atmosphere may have wider significance than is suspected at present. Considerable emphasis has been placed on the symbiosis between nitrogen-fixing bacteria, especially *Azotobacter*, and algae by means of which the algae are thought to supply the nitrogen-fixing bacteria with the carbohydrates necessary for the fixation process and to receive nitrogenous compounds in return. This hypothesis, however, has so far not been supported by adequate experimental proof. Since many of the soil algae lead a heterotrophic existence, they may, to some extent, compete with the other soil microorganisms for the available organic compounds.

On the whole, the algae have received relatively little attention as compared to the other soil organisms. Despite the fact that the literature on soil algae is extensive, the available information must still be considered as fragmentary. Little is known of the effect on the algal population of the soil of such important environmental factors as season of year, pH value of soil, moisture and organic matter contents, and the interrelationships of algae and other soil microorganisms with which they are in constant contact. The following experiments were designed with a view to obtaining answers to these questions.

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

EFFECT OF FERTILIZER TREATMENT AND SEASON OF YEAR

An investigation of the effect of fertilizer treatment and season of year on algae and other microorganisms was made on some of the soils of the experimental plots at the N. J. Agricultural Experiment Station. These plots had been used for 30 years for comparing different sources of nitrogen in a 5-year rotation of corn, oats, wheat, and 2 years of timothy. During the period of this experiment, corn was growing. The soil is Sassafras loam which had been neglected previous to 1908. The *A* plots have been without lime during the entire period. The *B* plots have been limed at the rate of 2 tons to the acre, ground limestone or ground oyster shells being applied at intervals of 5 years. The particular plots chosen for this experiment had received the following fertilizer treatments:

PLOT NUMBER	TREATMENT	A UNLIMED	B LIMED
		pH	pH
5	Minerals* and cow manure†	5.09	6.36
7	Nothing	4.33	6.26
11	Minerals and ammonium sulfate‡	3.88	5.27

* Superphosphate 320 pounds and muriate of potash 160 pounds per acre.

† 16 tons per acre.

‡ Equivalent to 320 pounds of nitrate of soda.

At stated intervals, extending from June 24, 1938 to January 25, 1939, these plots were sampled and analyzed for numbers of algae, bacteria, actinomycetes, and fungi. The methods used were as follows: Three samples were taken from each plot to a depth of 6 inches. These were sieved and thoroughly mixed. The algae in the soil samples obtained on June 24 were counted by means of the method recommended by Bristol Roach (13). This method was found to be extremely tedious and cumbersome, because of the large numbers of dilutions required for each count. The dilution method of Skinner (12), making use of the algal medium of Bristol Roach, was then tried and found to be satisfactory. The method requires three dilutions of the soil, the degree of dilution depending upon the number of algae likely to be present in the soil. As a rule, dilutions of 1-1000, 1-10,000, and 1-100,000 were found to be adequate. Ten replicate 1-cc. portions of each of the three dilutions were inoculated into test tubes containing 5 to 10 cc. of sterile algal medium. The cultures were then incubated in the light: during the first part of the experiment, under electric lights; later, in a greenhouse. After incubation for a month or longer, the tubes in which algal growth had appeared were recorded, and from these data the probable numbers of algae per gram of soil were calculated by means of the probability tables of Halvorson and Ziegler (9).

Egg-albumin agar was used for plating bacteria and actinomycetes, and peptone-glucose acid agar for fungi.

In order to simplify the discussion of the results, a composite table (table 1) was made of the pertinent data. The counts of fungi were not included, since they contributed little of special significance. Generally, fungi were present in greater abundance in the unlimed plots than in the limed. An examination of the results indicated that nothing could be gained by considering the bacteria and actinomycetes separately, and, therefore, their numbers were added together and reported as a single value in the table.

Liming had apparently little effect on the numbers of algae in plot 5B as compared to plot 5A. The soil samples taken from plot 5A on June 24, August 4, October 31, and January 25 contained a somewhat greater algal population than those taken from plot 5B, whereas 5B contained a slightly greater number of algae than 5A at the remaining two periods of sampling. The algal counts for both plots on June 24 and August 4 were too low to warrant comparison. Exclusion of these two periods leaves only the fourth period dur-

TABLE 1
Effect of fertilizer treatment and season of year on numbers of algae and other microorganisms in soil

Numbers per gram of oven-dry soil												
SOIL.....	5A		5B		7A		7B		11A		11B	
Date	Algae	Bact. + Act. $\times 10^6$	Algae	Bact. + Act. $\times 10^6$	Algae	Bact. + Act. $\times 10^6$	Algae	Bact. + Act. $\times 10^6$	Algae	Bact. + Act. $\times 10^6$	Algae	Bact. + Act. $\times 10^6$
June 24	174	5.8	32	4.5	4,792	1.3	2,357	5.7	6,034	2.4	28,327	3.7
Aug. 4	7,711	8.2	3,000	10.2	6,458	1.4	>100,000	10.8	14,400	2.2	>100,000	3.1
Aug. 30	18,800	8.2	18,900	9.7	18,900	3.1	33,150	6.2	3.1	6.5
Oct. 31	23,650	15.6	11,200	12.4	17,700	1.5	38,100	10.8	8,900	2.2	11,600	11.5
Dec. 13	59,000	6.2	62,500	11.4	85,400	1.9	212,000	8.1	>230,000	3.9	144,200	18.3
Jan. 25	37,200	10.6	26,200	12.2	85,400	2.5	110,400	4.0	13,400	...	>230,000	10.5

ing which soil 5A, with 23,650 algae per gram, had a distinctly higher flora than soil 5B, which contained 11,200 algae per gram. Liming, also, had no clear cut effect on the numbers of bacteria and actinomycetes present in these soils.

In the case of plots 7A and 7B, which had received no fertilizer or manure, the effect of liming was marked. Except for the first period, when the numbers of algae in both plots were low, the plot receiving lime (7B) had a distinctly greater algal population than the unlimed plot (7A). The difference between the two plots was most marked on August 4 when the number of algae in plot 7B was over 100,000 per gram, as compared to 6,458 per gram of soil in plot 7A, and on December 13 when there were 212,000 algae per gram of soil 7B and only 85,400 per gram of soil 7A. Liming, also, greatly affected the bacterial numbers; these were much higher in the limed plots at all periods of sampling.

Plots 11A and 11B, which had received minerals and $(\text{NH}_4)_2\text{SO}_4$, showed

differences similar to those observed in plots 7A and 7B. The limed plots, generally, had a greater algal population than the unlimed plots. This was clearly indicated on June 24, August 4, and January 25, when the algal population in soil 11B was about 4 to 20 times as great as that in soil 11A. The only time this relationship was reversed was on December 13. At this time, although the algal counts in both plots were high, soil 11A contained more than 230,000 algae per gram as compared to 144,200 algae in soil 11B. Liming was also beneficial to the bacterial population, since, in all cases, higher counts were obtained from plot 11B than 11A.

To compare the effect of fertilizer treatment on the algal and bacterial populations of the various plots, it is best to compare the unlimed and limed plots separately. On examining the numbers of algae from the unlimed plots it becomes evident that in five examinations, either soil 7A, which had received no fertilizer, or 11A, which had received minerals and $(\text{NH}_4)_2\text{SO}_4$, had a greater algal population than 5A, which had received minerals and cow manure. The only exception occurred on October 31, when plot 5A contained a somewhat higher algal population than 7A and 11A. The generally smaller algal population of soil 5A may be due to the added organic matter, which may, as will be shown in a later experiment, exert an inhibitory effect on algal development. There were no consistent differences between soils 7A and 11A.

The algal population of soil 5B was also much smaller than that of 7B or 11B, in practically all cases. These differences were greater than in the unlimed plots. For example, on January 25 soil 5B contained 26,200 algae per gram as compared to 110,400 in 7B and more than 230,000 in 11B. As was observed in the unlimed plots, there was no consistent difference between the numbers of algae in soils 7B and 11B.

In the unlimed plots there was an inverse relationship between the algae and bacteria (including actinomycetes). Soil 5A, with the smallest algal population, had the greatest number of bacteria and actinomycetes. In the limed plots, however, this relationship did not hold, since soil 5B, with a much smaller algal population than either soil 7B or 11B, did not contain significantly more bacteria and actinomycetes.

Temperature and moisture are very important factors in the development of algae, just as they are in the growth of other microorganisms. Insofar as the season of the year reflects a certain status of these two important environmental factors, it exerts a profound effect on the development of algae in the soil. Much more data than those presented here would be necessary before an attempt could be safely made to correlate the numbers of algae found at any one time in the soil with the season of the year. A few indications to be derived from the present data, however, are particularly suggestive.

The algal population was greater in all plots on August 4 than on June 24. Marked increases were found, especially in plots 7B and 11B, which contained more than 100,000 algae per gram at the later date, as compared to the 2,357 and 28,327, respectively, found on June 24. An examination of the official

weather reports for this period showed that, during the month of July, rain fell on 18 days and the total precipitation for the month was 7.77 inches, 4.05 inches above normal. This may well account for the larger algal populations on August 4, since the moisture conditions preceding the sampling period had been particularly favorable for algal growth. Another sharp increase in numbers of algae was observed on December 13. Large increases occurred in all of the plots. The weather reports show that, on November 12, 6 inches of snow fell. This blanket of snow, by keeping the soil moist and warmer than the surrounding atmosphere, probably created conditions favorable for algal development. On January 25, all the plots were covered with about 6 inches of snow; underneath this layer of snow, the soil was moist and algal population in most of the plots was higher than during the first four periods of sampling.

These results suggested the advisability of a closer study of the effect of moisture, pH, and organic matter on soil algae, under carefully controlled experimental conditions.

INFLUENCE OF MOISTURE

Although it is generally recognized that moisture is an important factor in the development of soil algae, very little direct experimental evidence is available concerning this effect. Esmarch (7) attributed the greater number of *Cyanophyceae* in cultivated soils than in uncultivated soils to the fact that cultivation, by improving the physical structure of the soil, brought about better nutritive and moisture conditions for algal growth. Bristol (3) studied the effect of desiccation upon algae. Soils obtained from arable land and from old gardens were spread out to dry in a warm room for at least a month. Despite the long period of desiccation, the soils were found to contain 64 species and varieties of algae consisting of 24 species of *Cyanophyceae*, 20 species of *Chlorophyceae* and 20 species of *Bacillariae* (diatoms). Bristol (2) also reported that she was able to isolate a species of blue-green algae, 4 grass-greens, and 1 diatom from dry soil stored for about 40 years. Fritsch (8) pointed out that a striking characteristic of terrestrial algae is the capacity of the ordinary vegetative cells, without any marked change and without special thickening of the walls, to withstand drought. Moreover, the change from the resting to the active condition was found to occur in a very short time, apparently because the terrestrial algae required only small amounts of moisture to replace that lost by the protoplast in drying.

Although these investigations indicate that many soil algae are resistant to desiccation, they give no idea as to how algae respond to varying moisture levels in soils. That the moisture content of the soil is an important factor in algal development was indicated in the previous experiment on the algal flora of the experimental plots, since periods of rain or snow brought about sharp increases in the numbers of algae. Further enlightenment on this question was sought in the following experiment.

Soil from plot 7B was air-dried, sieved, and placed, in 1-kgm. quantities, in

each of six suitable glazed earthenware pots. One pot of soil was left untreated; the moisture levels of the soils in the remaining five pots were adjusted to 20, 40, 60, 80, and 100 per cent of the moisture-holding capacity; that of the original soil was 36.6 per cent. The pots were covered with glass to keep out atmospheric contaminants and incubated in the laboratory at room temperature under electric lights. Calculated amounts of water were added each day to compensate for losses of moisture due to evaporation.

Within 6 days, a surface growth of algae became visible macroscopically on the soils brought to 40, 60, and 80 per cent of the moisture-holding capacity. Of these three soils, the first appeared to have the most abundant algal growth; and the last, the least. There was no apparent algal growth on the surface of the other soils. After 8 days' incubation, the relative growth in the differently treated soils was the same as after 6 days, but it was more abundant. After 13 days' incubation, the surface of the soil in each pot to a depth of 1

TABLE 2
Effect of moisture upon growth of soil algae
Numbers per gram of dry soil

SATURATION OF SOIL.....per cent	AIR-DRY	20	40	60	80	100
<i>Surface soil</i>						
Bact. + Act., $\times 10^6$	10.0	11.8	14.0	18.2	26.8	6.3
Algae.....	43,400	58,400	500,000	548,000	325,000	67,500
<i>Subsurface soil</i>						
Bact. + Act., $\times 10^6$	9.1	..	15.0	19.2	14.1	15.7
Algae.....	..	46,000	270,000	300,000	170,000	110,400

cm. and the subsurface soil were analyzed for numbers of algae, bacteria, and actinomycetes by the usual methods. The results are given in table 2.

The results for the surface 1 cm. of soil show clearly that the optimum moisture range for algal development lies between 40 and 60 per cent of the moisture-holding capacity. The greatest number of bacteria and actinomycetes was found in the 80 per cent soil. On either side of the optimum range, smaller numbers of algae and usually also of bacteria and actinomycetes were found. At 100 per cent saturation, the soil contained about the same number of algae as the air-dry soil. There was a smaller difference in numbers of algae between 60 and 80 per cent saturation than between 40 and 20 per cent saturation, which indicates that moisture in excess of the optimum is less detrimental to algal growth than are moisture levels below the optimum range.

In the subsurface soil, the same relationships held true as in the surface soil; the optimum range for algal development was between 40 and 60 per cent saturation, although the total numbers of algae were smaller than in the surface soil. The numbers of bacteria and actinomycetes were also greatest within

this range. At moisture levels on either side of the optimum range, the soil contained fewer microorganisms.

EFFECT OF pH

It is reasonable to expect that the pH of a soil will influence the growth of its algal flora, just as it is known to affect the other microorganisms in soil. Petersen (11) has reported, for example, that the algal flora in acid soils is different from that found in alkaline or neutral soils. It was also previously shown that the experimental plots which had received lime, have, as a rule, a more abundant algal flora than the unlimed plots.

In the following experiment, cultivated Sassafras loam having a pH of 4.2 was air-dried, sieved, and distributed in 200-gm. quantities into a series of six tumblers. One was left as control, and the remainder were treated with varying amounts of $\text{Ca}(\text{OH})_2$ in order to bring them to different pH levels. All

TABLE 3
Effect of pH upon the growth of algae in soil

Ca(OH) ₂ ADDED gm.	INITIAL pH	SURFACE GROWTH ALGAE	SUBSURFACE GROWTH*	
			Algae	Bact. + Act. $\times 10^6$
None	4.2	+	19,700	2.2
0.123	5.3	++	17,000	1.9
0.245	5.9	+++	120,000	5.5
0.318	6.3	+++	14,000	7.0
0.392	7.4	++++	34,600	14.2
0.490	7.6	++++	34,900	23.4

* Numbers per gram moist soil.

soils were brought to the optimum moisture content with distilled water, covered with glass to keep out contaminations, and incubated under artificial light. Water was added each day to compensate for evaporation.

After 2 months of incubation, the differences in the surface algal growths in the various tumblers were so apparent, macroscopically, and in some cases so luxuriant that they were recorded by direct observation rather than by the culture method. The subsurface soils, however, were analyzed for algae, bacteria, and actinomycetes by the usual methods. The results are recorded in table 3.

The surface growth of algae was greatly affected by the pH of the soil. At a pH of 4.2 there was only scant development of algae. As the pH of the soil was raised to 7.6 a marked gradual increase in algal growth was observed. At pH 7.4 and 7.6, a very luxuriant algal development took place.

In the subsurface soil, the effects of altering the pH were irregular. Although the soil at pH 5.9 contained many more algae than at pH 4.2, the rest of the soils at the higher pH levels showed little or no increases in numbers of

algae. Since the algae in the subsurface soil must depend upon preformed organic materials for their nutrition, lack of an adequate food supply was probably a limiting factor, despite the favorable reaction of the medium. The numbers of bacteria and actinomycetes increased greatly as the pH of the soil was raised.

These results tend to support the previous findings on the soils of the experimental plots that liming may have a marked beneficial effect upon the growth of algae as well as of bacteria and actinomycetes.

EFFECT OF ORGANIC MATTER

It is a well known fact that most algae, in the absence of light and in the presence of organic compounds, are able to live heterotrophically. One may, therefore, expect that the addition of organic matter to soil will influence the algal population, either directly or indirectly, by affecting the remaining microbial flora with which the algae would compete for food. The following experiment was outlined in order to obtain some understanding of the influence of organic matter on the algal flora of soil and the possible related influences to the development of other soil microorganisms.

Unfertilized but limed soil from plot 7B was used. The air-dry, sieved soil was placed, in 1200-gm. amounts, into each of 16 glazed earthenware pots. The pots were divided into two series, each consisting of eight different treatments, as follows:

Pot Number	Treatment
1	Nothing added
2	Dextrose, 1 per cent
3	Cellulose, 1 per cent
4	Cellulose, 1 per cent + 21 mgm. nitrogen as $\text{NH}_4\text{H}_2\text{PO}_4$
5	Alfalfa, 1 per cent
6	Straw, 1 per cent
7	Straw, 1 per cent + 21 mgm. nitrogen as $\text{NH}_4\text{H}_2\text{PO}_4$
8	Manure, dry, 1 per cent

The organic materials were thoroughly mixed with the soil, and sufficient distilled water was added to bring the soil to the optimum moisture level. The pots were covered with glass to prevent external contamination. One set was placed in the dark in a 28°C. incubator, in order to study algal development under purely heterotrophic conditions; the algae were thus placed in direct competition for nutrients, including energy supply, with the other heterotrophic microorganisms. The other set was placed in a greenhouse and exposed to diffuse daylight; under these conditions, the algae could develop as autotrophs on the soil surface, whereas organic compounds were still necessary for the growth of algae in the subsurface soil. At weekly intervals, the pots were weighed, and water equal to the amount of moisture lost by evaporation was added. The variously treated soils were analyzed for abundance of algae, bacteria, and actinomycetes by the usual methods. The soil was re-

moved from each pot, thoroughly mixed, and sampled. Figure 1 represents the growth of microorganisms in the light; and figure 2, in the dark.

Growth in the light

In the light, the number of bacteria and actinomycetes in the control soil remained at a fairly constant level throughout the incubation period, though slightly above the number originally present. The algae, on the contrary, increased greatly and, after 35 and 179 days, 230,000 per gram of soil were present as compared with the initial number of 27,500.

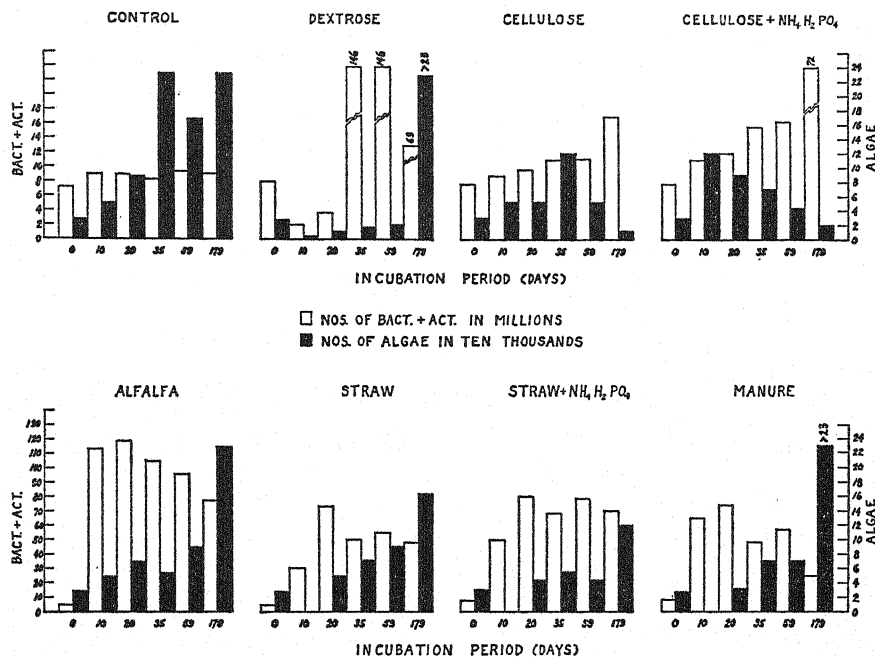


FIG. 1. EFFECT OF ORGANIC MATTER UPON DEVELOPMENT OF ALGAE AND OTHER MICRO-ORGANISMS IN SOIL IN THE LIGHT

In the soil containing dextrose, although for some unknown reason the increase in numbers of bacteria and actinomycetes was much slower than might have been expected, high counts of 146×10^6 per gram were obtained after 35 and 59 days. After 179 days, the count had dropped to 63×10^6 . Up to the last incubation period, growth of algae was repressed, the numbers being less than at the beginning. A tremendous increase in algae was found after 179 days; this accompanied a sharp decrease of bacteria and actinomycetes.

The soil treated with cellulose showed a gradual increase in the numbers of bacteria and actinomycetes. The algae also increased during the early periods

of incubation, but later, as the bacteria and actinomycetes continued to multiply, the algal numbers decreased until, after 179 days, fewer algae were present in the soil than at the beginning of the experiment.

The soil treated with cellulose and nitrogen also showed an increasing population of bacteria and actinomycetes throughout the incubation period. Their numbers were somewhat higher than those in the soil which received cellulose alone, undoubtedly because of the favorable effect of the added nitrogen. In the beginning, the algae also increased to a greater extent than in the soil treated with cellulose alone. As the bacteria and actinomycetes continued to

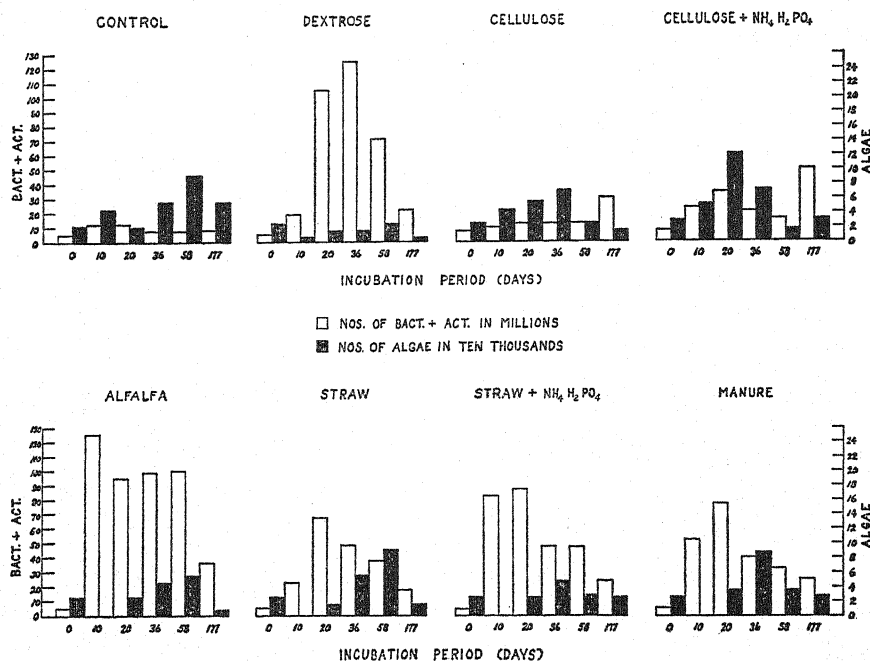


FIG. 2. EFFECT OF ORGANIC MATTER UPON DEVELOPMENT OF ALGAE AND OTHER MICRO-ORGANISMS IN SOIL IN THE DARK

increase, the algal population decreased, and at the end of the incubation period, it was at about the original level.

The addition of alfalfa brought about the development of large numbers of bacteria and actinomycetes. After 20 days of incubation and until the end of the experiment, a gradual decrease occurred. The algae, as a rule, increased continuously, and the greatest number, 230,000 per gram of soil, was present at the conclusion of the experiment. This was no doubt due to an increase in the available nitrogen and minerals, resulting from the decomposition of the alfalfa.

The soil treated with straw showed, at first, an increasing population of

bacteria and actinomycetes, which later declined somewhat. The algae increased regularly, and at the end of the experiment, about a sixfold increase over the number originally present in the soil was found.

The addition of straw and nitrogen resulted in the production of a microbial sequence similar to that which occurred when straw alone was added to the soil, but the numbers of bacteria and actinomycetes were somewhat higher and those of algae somewhat lower. The algae increased slowly, the greatest number being present at the end of the experiment.

The addition of stable manure to soil brought about an increase of bacteria and actinomycetes. After 20 days, the numbers of these two groups of organisms decreased. The algal population increased continuously, and more than 230,000 per gram of soil were present after 179 days of incubation.

These results can be interpreted as follows. In the control soil, the heterotrophic microorganisms failed to multiply appreciably because the supply of available food was limited. The algae, on the other hand, being able to produce their own food photosynthetically on the surface of the soil, were not so limited and, therefore, multiplied. The addition of dextrose to the soil brought about a profound change in the relation between the heterotrophic and the autotrophic microorganisms. With an abundant supply of food (dextrose) available to the algae as well as to the heterotrophs, the latter increased tremendously, whereas the growth of the algae was greatly repressed. Since conditions of light, temperature, and moisture were the same as in the control soil, the inhibition of algal growth appears to be due to some factor, possibly nitrogen, which in this case became limiting as a result of the extensive growth of bacteria and actinomycetes. This is supported by the fact that the algae increased rapidly when the heterotrophic organisms had decreased appreciably, and also by the results obtained from the soils to which cellulose and cellulose plus nitrogen had been added. In the latter cases, during the early periods of incubation, the bacteria and actinomycetes did not multiply to a very great extent. Conditions were, therefore, similar to those in the control soil, and as a result the algae multiplied. As the bacteria and actinomycetes continued to increase in numbers, however, a condition resembling that in the soil treated with dextrose resulted, and the algal population decreased.

In the soils that received alfalfa, straw, straw plus nitrogen, and manure, the algal population, as a rule, increased continuously throughout the incubation period, whereas the bacteria and actinomycetes increased to high levels at first, but later declined somewhat. These results were in contrast with those obtained in the previous soils in which extensive multiplication of heterotrophic organisms markedly restricted algal growth. If it is assumed that nitrogen became the limiting factor for the growth of algae in the presence of actively multiplying bacteria and actinomycetes, then most of these results can be readily explained. It is well known that alfalfa and manure have a high enough nitrogen content so that during microbial decomposition, nitrogen is liberated. In those soils, therefore, that received these organic materials,

despite the extensive growth of bacteria and actinomycetes, the algae continued to multiply since nitrogen was always available. Sufficient nitrogen was probably also present in the soil receiving straw plus nitrogen, and, in this case, algal growth was not inhibited. In the soil that received straw alone, the algae multiplied continuously. Although one can expect that the addition of straw will bring about a condition of nitrogen starvation in soil, yet the rate of decomposition of this material is relatively slow as compared, for example, with the decomposition of dextrose. The small amounts of nitrogen that probably were available at any one time may have been sufficient for the algal growth produced. Most of the algal development occurred when the numbers of bacteria and actinomycetes were decreasing, a process which undoubtedly resulted in the liberation of some available nitrogen. It is also possible that, in all of the treated soils, competition for other essential elements, such as phosphorus and potassium, and the formation of toxic substances by the other microorganisms may have played a part in limiting the growth of algae.

On the whole, the control soil which had not received any organic matter had a greater algal population than those soils which had received the various types of organic matter. There are some indications that the residual effects of organic matter decomposition favored algal development, since, after 179 days, the soil that received dextrose and manure had a greater number of algae than the control soil.

Growth in the dark

The results presented in figure 2 substantiate the fact that algae can multiply to some extent in the dark, as has been shown by Skinner (12). Most of the increases, however, were of a much smaller order of magnitude than that which occurred in the soils kept in the light where photosynthetic activity was possible. The addition of dextrose repressed algal growth as it did in the soil kept in the light, with the important difference that in the dark, the algae did not increase after the numbers of bacteria and actinomycetes had declined. The same phenomenon was noted in the soils treated with alfalfa, straw, straw plus nitrogen, and manure. In the soils treated with cellulose and cellulose plus nitrogen, low numbers of algae appeared to be correlated with high numbers of bacteria and actinomycetes as was the case in the light.

These facts indicate that a lack of an adequate food supply, as well as competition, in some cases, for nitrogen or other essential elements limited the growth of algae in the dark even though various types of organic matter were present. If the added organic materials or the decomposition products formed from them through the activity of the bacteria, actinomycetes, and fungi were readily available as food sources for the algae, marked increases in numbers should have occurred at some time during the incubation period. Such increases, however, did not occur. On the whole, there was little difference between the numbers of algae in the control soil and in the soils that received the organic materials. Where small differences occurred, the smaller numbers of

algae were to be found, mainly, in the treated soils. These results make it seem doubtful that heterotrophic nutrition is an important factor in the development of algae in soil. There are too many other microorganisms in the soil that are better adapted than the algae to this type of nutrition. The ability to utilize organic matter to a small extent may enable the algae to exist in the subsurface soil, but significant activity probably does not occur except at the very surface where light is available for photosynthesis, which appears to be essential for extensive algal development.

It is interesting to observe that the inhibitory effect of organic matter on the algal population as noted in this experiment parallels the previous observations on the field soils, where it was found that fewer algae were present in the manured soils than in the unmanured.

SUMMARY AND CONCLUSIONS

In the experimental plots it was found that, with the exception of the plot receiving stable manure, liming had a definitely beneficial effect upon the development of algae as well as of bacteria and actinomycetes. The plots receiving stable manure and minerals had smaller algal populations than the plots which did not receive any treatment or those that received minerals and nitrogen in the form of ammonium sulfate. No consistent differences could be found between the latter two treatments. There were clear indications that after periods of rain or snowfall, the algal population of the plots increased as a result of more favorable conditions of moisture.

The amount of moisture in soil had a decided effect on the growth of the algae. The optimum moisture range for the growth of algae was essentially the same as that for the bacteria and actinomycetes, namely, 40 to 60 per cent of the moisture-holding capacity of the soil. An excess of moisture was less detrimental to algal growth than was suboptimum moisture. This applied to both surface and subsurface soils. A high moisture content of the very surface of the soil affected algal development more than did a similar moisture content below the surface, since it is at the surface that the algae are able to utilize radiant energy, whereas they must live as heterotrophs below the surface. This suggests that extensive development of algae on the soil surface is confined to special periods or special conditions and that there may be periods of rapid development followed by periods of relative inactivity.

As the pH of a soil was raised from 4.2 to 7.6 by the addition of $\text{Ca}(\text{OH})_2$, a marked, gradual increase in algal growth on the surface was noted. In the subsoil, the effects of altering the pH were irregular. The numbers of bacteria and actinomycetes increased greatly with increase in the pH of the soil.

In the presence of light, the addition of organic matter inhibited, partially or completely, algal growth during the period of active decomposition of the organic matter by the bacteria and actinomycetes. The extent of the effects differed with the type of organic matter added. Inhibition appeared to be due to competition for essential elements, largely nitrogen and possibly others,

which the bacteria and actinomycetes were able to utilize more rapidly than were the algae. Other factors such as unfavorable effects of specific organic substances, deficiencies of oxygen, and definite antagonisms, may also have played a part. After active microbial decomposition had slowed down and the bacteria and actinomycetes had begun to die off, extensive algal growth occurred.

In the absence of light, even though various types of organic substances were present, algal development was restricted because, under these conditions, at least two limiting factors were in operation: first, unfavorable competition for essential elements, at least in some cases; and second, lack of an adequate food supply, since the added organic substances and products resulting from their decomposition could be utilized by the algae either not at all or only to a small degree. Even after the bacteria and actinomycetes had decreased in numbers, the algae failed to multiply extensively, since they were still limited by a lack of available food.

REFERENCES

- (1) ALLISON, F. E., AND MORRIS, H. J. 1930 Nitrogen fixation by blue-green algae. *Science* 71: 221-223.
- (2) BRISTOL, B. M. 1919 On the retention of vitality by algae from old stored soils. *New Phytol.* 18: 92-107.
- (3) BRISTOL, B. M. 1920 On the algal flora of some desiccated English soils: an important factor in soil biology. *Ann. Bot.* 34: 35-80.
- (4) BRISTOL, B. M. 1923 Microorganisms of the Soil [by E. J. Russell et al.], chap. 6, p. 99-117. London.
- (5) DE, P. K. 1939 The role of blue-green algae in nitrogen fixation in rice fields. *Proc. Roy. Soc. [London]* (B) 127: 121-139.
- (6) DREWES, K. 1928 Über die Assimilation des Luftstickstoffs durch Blaualgen. *Centbl. Bakt.* (II) 76: 88-101.
- (7) ESMARCH, F. 1914 Untersuchungen über die Verbreitung der Cyanophyceen auf und in verschiedenen Boden. *Hedwigia* 55: 244-273.
- (8) FRITSCH, F. E. 1922 The moisture relations of terrestrial algae: I. Some general observations and experiments. *Ann. Bot.* 36: 1-20.
- (9) HALVORSON, H. O., AND ZIEGLER, N. R. 1932 Application of statistics to problems in bacteriology. A means of determining bacterial population by the dilution method. *Jour. Bact.* 25: 101-121.
- (10) HARRISON, W. H., AND AIYER, S. 1913, 1914 Gases of swamp rice soils. *India Dept. Agr. Mem., Chem. Ser.* 3: 65-106 (1913); 4: 1-17 (1914). [Cited by S. A. Waksman in *Principles of Soil Microbiology*, ed. 2, p. 219. 1932.]
- (11) PETERSEN, J. B. 1915 Danske aerofile alger. *K. Danske Vidensk. Selsk. Skr. 7 Raekke, Naturv. og Math. Afd.* 12: 7. [Cited by S. A. Waksman in *Principles of Soil Microbiology*, ed. 2, p. 208. 1932.]
- (12) SKINNER, C. E., AND GARDNER, C. G. 1930 The utilization of nitrogenous organic compounds and sodium salts of organic acids by certain soil algae in darkness, and in light. *Jour. Bact.* 19: 161-179.
- (13) WAKSMAN, S. A., et al. 1927 Methoden der mikrobiologischen Bodenforschung *Abderhalden's Handbuch*, Abt. II, Teil 3: 715-864.

BACTERIOLOGICAL STUDIES ON A NEW CAPSULATED BACILLUS, *BACILLUS KRZEMIENIEWSKI*

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A peculiar new capsulated bacillus was isolated from arable soil during soil fertility tests by the Winogradsky Azotobacter method (6). This bacillus grows rapidly at 28°C. on agar plates with nitrogen-free Ashby medium, forming large convex colonies resembling hemispherical glass beads (fig. 1). The colonies are tough and are difficult to separate from the medium. Microscopic preparations show the presence of sporulating bacilli enclosed in large well-defined and separate capsules.

There are numerous descriptions of capsulated bacteria in the literature, and, in fact, nearly all bacteria produce some polysaccharide capsular substance. Only two brief notes, however, refer to bacteria which might be similar to the organism just isolated from soil. Krzemieniewski (5) when studying Azotobacter also saw colonies of some bacteria which he described as follows: "Neben

diesen konnte man stets stark wachsende, sphärisch-konvexe fast wie Wassertropfen durchsichtige Kolonien beobachten." Winogradsky (7) also isolated from soil a bacterium (*Bacille gommeux*) very similar to the one described herein, with the difference that the organism isolated by him produced a uniform slime in which were distributed the capsulated bacilli, whereas the capsules of *Bac. Krzemieniewski* are well defined and clearly visible in microscopic preparations. Much of the work on capsulated bacteria and the biochemistry of the capsular substance has been reviewed, among others, by Buchanan and Fulmer (3) and by Mikulaszek (6). Greig-Smith (4), who studied a number of gum-producing organisms, has suggested that these capsulated bacteria may be of importance in the microbial processes in soil.

DESCRIPTION OF BAC. KRZEMIENIEWSKI

Morphology

The size of the cells without capsules is 3.0–4.0 x 1.0 μ , with capsules 6.0–7.5 x 5.5–6.0 μ . In older cultures the spores are numerous and after formation are enclosed, at least for a time, within the capsules. The size of the spores is almost constant, 2.0 x 1.0 μ (fig. 2).

¹ During the course of these investigations, A. G. Norman was at the Rothamsted Experimental Station.

Staining

These organisms seem always to be Gram-negative irrespective of the nature of the medium. This is somewhat unusual, inasmuch as spore-forming organisms are commonly Gram-positive. The vegetative cells rarely stain uniformly. Usually there are one or two easily stained transverse chromatin-like bands in each cell (fig. 3). If stained by the Neisser method for volutine, small deeply stained granules appear in place of bands (fig. 4). With Giemsa stain the protoplasm appears blue, the bands dark blue, and the capsule light pink. The capsules are always well defined and stain distinctly with dyes (fig. 5). The chromatin bands appear to participate in cell division, and as the first stage in division, the band which is usually centrally placed splits in two lengthwise, and each half goes to a young cell (1).

Growth

The growth on solid media containing easily available sources of carbon and only traces of organic or mineral nitrogen is very distinctive. Large hemispherical colonies, 5–10 mm. in diameter, appear in form resembling glistening glass beads (fig. 1). The colonies are firm but resilient and adhere strongly to the medium. On liquid Ashby medium the growth is very poor, but the addition of sterilized soil solution results in rapid growth and the production of much capsular substance. The addition of 20–25 per cent of soil extract to either solid or liquid Ashby medium appeared to promote the best conditions for growth. The mannitol in the Ashby medium may be satisfactorily replaced by glucose and several other carbohydrates. The colony appearance on other media is less distinctive, as follows:

- On nutrient agar slants, gelatinous growth.
- On agar stab tubes, growth only on the surface.
- On nutrient broth, thick gelatinous scum is formed.
- On peptone water, growth similar to that on nutrient broth.
- Gelatine stab, mucilaginous growth on surface and liquefaction of gelatine.
- On potato, grayish fluid growth, microscopic examination shows absence of typical capsules and apparent degeneration of cells with some spirillum-shaped forms (fig. 6).
- On milk, very slow growth, coagulation without formation of acid, very slow peptonization.

Some variation in the extent of formation of capsules was observed on different media, the carbon and nitrogen sources being changed.

As a basic medium the following mineral solution was used: 0.5 gm. K_2HPO_4 , 0.2 gm. $MgSO_4$, 0.2 gm. $NaCl$, and 1 liter of tap water. Mannitol (1 per cent) was the carbon source employed when different sources of nitrogen were tested. The following nitrogen sources were used: KNO_3 , KNO_2 , $(NH_4)_2SO_4$, peptone, asparagine, broth, horse blood serum. Since it was observed that this bacillus grows well on media containing small quantities of nitrogen, all the nitrogen sources tested were added in two concentrations, 0.1 and 0.01 per cent. One-

hundred-milliliter round flasks were filled with 50 ml. of medium, sterilized, and inoculated with two drops of culture grown on a medium containing asparagine as source of nitrogen. The inoculated media and all controls were incubated for 12 days at 28°C. The viscosity of the culture was taken as a measure of the growth and formation of bacterial slime (2). This was determined by recording the time necessary for the delivery of the first 5 ml. of culture from a 10-ml. standard pipette (table 1).

TABLE 1
*Influence of different nitrogen sources on the formation of capsular material by
Bac. Krzemieniewski*

SOURCE OF NITROGEN AND PERCENTAGE CONCENTRATION	AVERAGE TIME OF DELIVERY OF 5 ML.	
	Strain A	Strain B
	<i>seconds</i>	<i>seconds</i>
KNO ₃ { 0.1.....	2.0	2.0
	0.01.....	2.4
KNO ₂ { 0.1.....	2.0	2.1
	0.01.....	3.1
(NH ₄) ₂ SO ₄ { 0.1.....	2.0	2.0
	0.01.....	3.4
Asparagine { 0.1.....	3.7	4.2
	0.01.....	2.4
Peptone { 0.1.....	3.6	2.7
	0.01.....	2.4
Broth { 1.0.....	2.4	2.4
	0.1.....	2.9
Blood serum { 0.1.....	2.2	2.0
	0.01.....	2.0
No added nitrogen, inoculated.....	2.5	2.2
No added nitrogen, uninoculated.....	2.0	2.0

In one of the flasks to which nitrate had been added, the bacillus (original strain A) lost its capsulated habit, and even after several transfers into media more suitable for the formation of capsules, these did not again appear. Growth of this new variety (strain B) as affected by different nitrogen sources was measured in the same way as that of strain A.

With strain A, 0.01 per cent KNO₂, 0.1 per cent asparagine, and 0.1 per cent peptone gave the highest viscosity; in some cases growth and viscosity were less than in the inoculated control flasks without added nitrogen. Strain B behaved somewhat differently from strain A.

Agglutination experiments with strain B without capsules gave results similar to those with the original capsulated strain A, since antisera produced by immunization of rabbits with strain B, as well as those produced by immunization with strain A, agglutinated both strains. In all cases the new strain without capsules agglutinated more strongly, that is, at higher dilutions of antisera, than the original strain (table 2).

The effect of different carbon sources was tested in the same way as that of nitrogen sources, the basic medium being the salt solution already described, to which was added 0.01 per cent peptone as nitrogen source. The following carbon sources were added at a concentration of 1 per cent: starch, dextrin, sucrose, lactose, maltose, dextrose, levulose, arabinose, xylose, mannitol, and glycerol. A different pipette was used for the determination of rate of flow, and the delivery time for 5 ml. of uninoculated control medium was 6.6 seconds (table 3).

TABLE 2
Results of agglutination experiments with Bac. Krzemieniewski*

ANTISERUM PRODUCED BY	NUMBER OF SERUM	AGGLUTINATION TITERS WITH	
		Strain A	Strain B
Strain A.....	1	1:400	1:800
	2	1:800	1:1600
	3	1:800	1:16000
Strain B.....	4	1:100	1:800
	5	1:800	1:1600
	6	1:400	1:800

* Agglutination of strain B was made with bacteria grown on a solid medium and suspended in physiological saline; agglutination of the original capsulated strain A was made, not with a suspension in physiological saline, but directly with a culture in the liquid medium. This applies also to immunization.

The best sources of carbon were the monosaccharides, levulose and dextrose. Next were the disaccharides, lactose and maltose.

As this organism appeared to grow without added nitrogen, the possibility arose that it might fix atmospheric nitrogen. Repeated experiments were made² to check the correctness of this assumption. The cultures were grown on media containing sucrose or mannitol as sources of energy. Preliminary experiments were made with liquid media in shallow layers in Erlenmeyer flasks, but it was found that solid media are more satisfactory. Cultures were therefore grown on carefully washed and sterilized sand saturated with the same media. In several tests traces (0.005 per cent) of sodium molybdate were added as a possible stimulant for the fixation of nitrogen. Nitrogen

² By S. F. Śnieszko, then at the Rothamsted Experimental Station as a Fellow of the Fundusz Kultury Narodowej.

determinations were made by the Kjeldahl method. In all, determinations were carried out on 25 cultures and 24 uninoculated controls incubated for 14 to 21 days at 28°C. The average results of the experiments are shown in table 4.

TABLE 3

Influence of different carbon sources on the formation of capsular material by Bac. Krzemieniewski

SOURCE OF CARBON IN CONCENTRATION OF 1 PER CENT	AVERAGE TIME OF DELIVERY OF 5 ML. STRAIN A
	<i>seconds</i>
Starch.....	12.1
Dextrin.....	13.7
Sucrose.....	11.7
Lactose.....	16.8
Maltose.....	16.8
Dextrose.....	17.4
Levulose.....	18.9
Arabinose.....	12.4
Xylose.....	13.4
Mannitol.....	12.2
Glycerol.....	8.5
Medium without carbohydrate, uninoculated.....	6.6

TABLE 4

Experiments on nitrogen fixation by Bac. Krzemieniewski

MEDIAN	CONTROLS, N FOUND	INOCULATED, N FOUND	GAIN OR LOSS OF N
	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Mineral liquid medium with 2 per cent mannitol...	0.20	0.25	+0.05
Mineral liquid medium with 2 per cent sucrose. . .	0.27	0.78	+0.51
Sand saturated with mineral medium with 2 per cent mannitol.....	0.98	1.56	+0.58
Sand saturated with mineral medium with 2 per cent sucrose.....	0.66	0.91	+0.25
Liquid mineral medium with 2 per cent sucrose and 0.005 per cent Na_2MoO_4	0.21	0.17	-0.04
Sand saturated with liquid mineral medium with 2 per cent sucrose and 0.005 per cent Na_2MoO_4 ..	0.93	1.47	+0.54
Liquid mineral medium with 2 per cent sucrose + 0.1 per cent $(\text{NH}_4)_2\text{SO}_4$	25.00	24.60	-0.40
Liquid mineral medium with 2 per cent sucrose + 0.1 per cent asparagine.....	22.55	20.50	-2.05

The magnitude of the differences obtained in these experiments was insufficient to justify any claim of nitrogen fixation.

SUMMARY

A new capsulated bacillus was isolated from cultivated soil. On solid media the colonies are large and raised, resembling hemispherical glass beads. The

capsules are large and distinct, and stain slightly with most dyes. Media containing more than traces of organic or inorganic nitrogen are not favorable for capsule formation. As judged by the increase in viscosity of the medium, levulose and dextrose are the best carbon sources, lactose and maltose also being good. It was suspected that this organism might fix nitrogen, but the differences in nitrogen obtained in inoculated and uninoculated media were not large enough to support this view.

This bacillus has been called *Bacillus Krzemieniewski* n.sp.

REFERENCES

- (1) ALLEN, L. A., APPLEBY, J. C., AND WOLF, J. 1939 Cytological appearances in a spore-forming bacillus. Evidence of meiosis. *Zentbl. Bakt.* (II) 100: 3-16.
- (2) ANDERSON, D. A. 1933 The production of gum by certain species of *Rhizobium*. *Iowa Agr. Exp. Sta. Res. Bul.* 158: 1-56.
- (3) BUCHANAN, R. E., AND FULMER, E. I. 1928, 1930 Physiology and Biochemistry of Bacteria, vol. 1 (1928), vols. 2, 3 (1930). Williams & Wilkins Co., Baltimore.
- (4) GREIG-SMITH, R. 1912 Bacterial slimes in soil. *Zentbl. Bakt.* (II) 34: 226-227.
- (5) KRZEMIENIEWSKI, S. 1908 Untersuchungen über *Azotobacter chroococcum* Beij. *Bull. Acad. Sci. Cracovie. Cl. Sci. Math. Nat.* 9: 929-1051.
- (6) MIKULASZEK, E. 1935 Bacterial polysaccharides. *Arch. Tow. Nauk. Lwów. Dz.* III. 6: 1-115.
- (7) WINOGRADSKY, S. 1926 Études sur la microbiologie du sol: II. Sur les microbes fixateurs d'azote. *Ann. Inst. Pasteur* 40: 455-520.

PLATE 1

GROWTH OF *BACILLUS KRZEMIENIEWSKI*

- FIG. 1. Colonies on Ashby agar plate. Slightly magnified.
FIG. 2. Cells on Ashby agar plate. $\times 1400$
FIG. 3. Cells on Ashby and soil extract liquid medium. $\times 1400$
FIG. 4. Cells on Ashby and soil extract liquid medium, Neisser stain. $\times 700$
FIG. 5. Cells on Ashby glucose medium, Giemsa stain. $\times 700$
FIG. 6. Culture on potato. $\times 1400$

FIG. 1

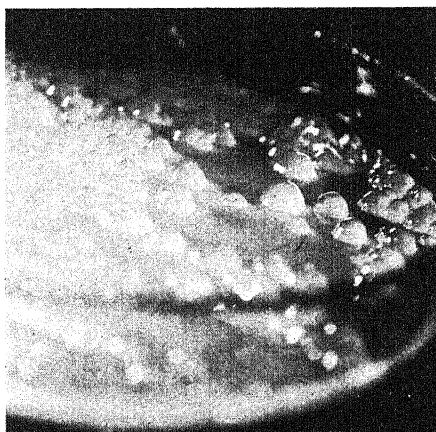


FIG. 2



FIG. 3



FIG. 5

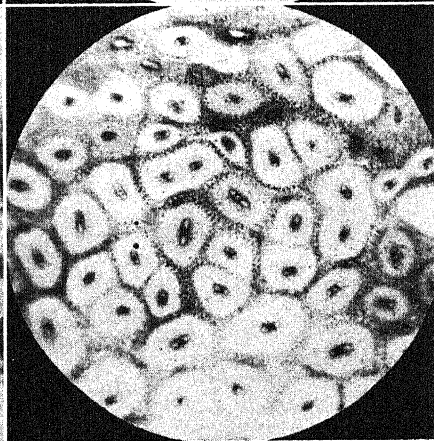


FIG. 4

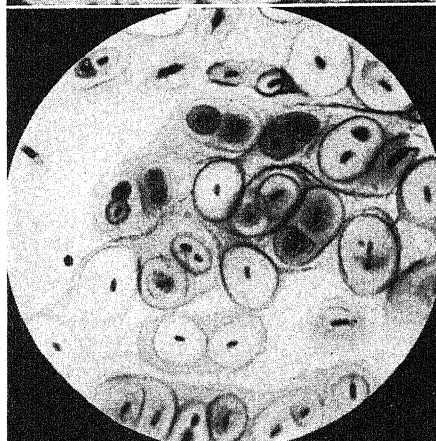
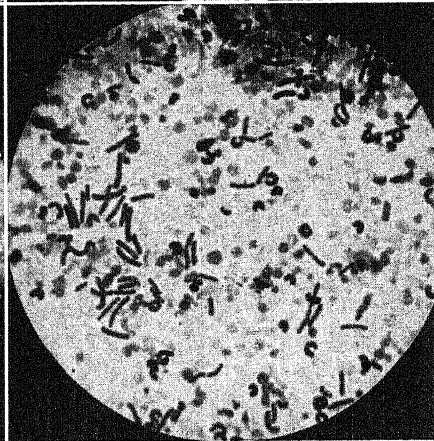


FIG. 6



EXPERIMENTS ON THE CHEMICAL NATURE AND PROPERTIES
OF THE POLYSACCHARIDE PRODUCED BY *BACILLUS*
KRZEMIENIEWSKI N.SP.

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A new microorganism producing gelatinous or mucilaginous growth was isolated from soil by Kleczkowska. Kleczkowska, Norman, and Snieszko² investigated some of the morphological and physiological properties of this organism, which they have called *Bacillus Krzemieniewski*. Its most characteristic property is the formation of thick, gelatinous capsules on various carbohydrate media. In this paper is described an investigation of the nature of the capsular material.

The capsular substance is easily obtained by precipitation with 75 per cent ethanol and appears as a fibrous, slightly grayish precipitate, swelling in water and becoming highly gelatinous. If it is precipitated directly from the culture media, the ash content is high, and the inorganic impurities cannot be removed by repeated reprecipitation with ethanol. Electrodialysis, however, reduces the ash content to less than 1 per cent and causes the polysaccharide to precipitate in the form of a fine cloudy suspension. Hydrolysis of the crude product liberates only 20–30 per cent reducing sugar, whereas 60 per cent is obtained from the electrodialyzed preparation. The content of both nitrogen and phosphorus is below 0.15 per cent.

A pure polysaccharide can be obtained in the form of a yellow viscous liquid by treatment of the crude product with 5 per cent potassium hydroxide, and may be precipitated by ethanol. Such preparations after electrodialysis give 96 per cent reducing sugar on hydrolysis.

The physical properties of this capsular polysaccharide from *Bacillus Krzemieniewski* indicate a large molecule, and chemical analyses are in accordance with the view that it is a polymannose, mannose having been identified by its optical rotation and by the formation of the typical hydrazone and osazone. The same polysaccharide was apparently produced in cultures developing on

¹ While carrying out this research, A. Kleczkowski was a Fellow of the Foundation for National Culture.

² Kleczkowska, J., Norman, A. G., and Śnieszko, S. 1940 Bacteriological studies on a new capsulated bacillus, *Bacillus Krzemieniewski*. *Soil Sci.* 49: 185–191.

either mannitol or glucose. A similar polymannose (manno-carolose) has been described as a product of the metabolism of *Penicillium charlesii*³.

EXPERIMENTAL

Preparation of crude product

Liquid cultures of *Bacillus Krzemieniewski* were concentrated *in vacuo* at a temperature of 70°C., and the polysaccharide was precipitated by addition of ethanol to give a concentration of 70 per cent. By repeated solution in water and reprecipitation the product was purified. After drying in a vacuum it was pulverized and treated with KOH.

Hydrolysis with potassium hydroxide

Five grams of powder were suspended in 300 ml. 5 per cent KOH and heated in a water bath for 3 hours under reflux. The solution obtained was mucilaginous and became more viscous on cooling. An insoluble precipitate, 75 per cent ash, was centrifuged off. The polysaccharide was precipitated by treatment again with ethanol. The precipitate was suspended in 800 ml. water and electrodialed.

Electrodialysis

The suspension was electrodialed for 4 days, cellophane films in a Pauli apparatus being used. The length of the chamber was 10 cm., the film surface 80 sq. cm., and the current 500 volts. During electrodialed the polysaccharide accumulated at the bottom of the chamber in the form of a cloudy precipitate and was coagulated by addition of ethanol to give 80 per cent concentration after resuspension in about 500 ml. water. The yield of pure product was 0.5 gm.

Analysis of carbon and hydrogen

Amount taken 0.01903 gm.....	CO ₂ = 0.03093 gm.	H ₂ O = 0.01055 gm.
(C ₆ H ₁₀ O ₅) _n calculated.....	C = 44.44 per cent	H = 6.22 per cent
found.....	C = 44.33 per cent	H = 6.15 per cent
	ash = 0.75 per cent	

Hydrolysis with sulfuric acid and identification of products

Hydrolysis was effected by treatment of the polysaccharide with 4 per cent H₂SO₄ for 1 hour at 120°C. The acid was neutralized by addition of barium hydroxide, the excess of which was removed by CO₂. The filtrate was concentrated *in vacuo*, and reducing sugar was determined by the micro Bertrand method. The optical rotation was determined, and phenylhydrazine and osazone were prepared.

³ Haworth, W. N., Raistrick, H., and Stacey, M. 1935 Polysaccharides synthesized by microorganisms: I. The molecular structure of manno-carolose produced from glucose by *Penicillium charlesii* G. Smith. *Biochem. Jour.* 29: 612-621.

Optical rotation: (Mannitol being the energy source in the culture medium) $[\alpha]_D = -13.8^\circ$ ($t = 17.9^\circ$); (glucose being the energy source in the culture medium) $[\alpha]_D = -13.9^\circ$ ($t = 17^\circ$).

The phenylhydrazone was prepared from 2 per cent aqueous solution and recrystallized twice, m. p. 197° . The osazone was characteristic of glucose and after purification had a m. p. of 208° .

SUMMARY

The nature of the capsular substance formed by *Bacillus Krzemieniewski* was investigated by preparation and analysis. It was found to be a polymannose composed of l-mannose units and from its physical properties may be assumed to have a high molecular weight.

ASSIMILATION OF PHOSPHORUS BY A MIXED SOIL POPULATION AND BY PURE CULTURES OF SOIL FUNGI¹

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An extensive literature has accumulated on the assimilation of phosphorus compounds by soil microorganisms, particularly the bacteria and fungi. Egorov (4) found phytin to be an excellent source of phosphorus for *Aspergillus niger* and *Penicillium glaucum* in sterile solution and suspected that there was an indirect assimilation of the phytin by fungi preceded by the splitting of this compound. Dox (2) and Dox and Golden (3) also obtained excellent growth of *Asp. niger* in Raulin's medium containing various organic compounds of phosphorus and suggested that phosphoric acid is first split off from the organic complex by means of some enzymes excreted by the mold and is then utilized in this form. According to Schnücke (9), the assimilation of phosphate by *Asp. niger* varies with the stage of growth and with the nitrogen source; the ratio of the amounts of phosphorus and nitrogen assimilated were believed to be more or less constant, the $P_2O_5:N$ being about 1:2. When the culture was left for a long time, the total phosphorus content of the fungus mycelium rapidly decreased, even though the actual weight of the mycelium did not diminish to a great extent.

Stoklasa (10) reported that *Azotobacter* contains 2.18 per cent phosphorus, 79 per cent of which is in the form of nucleic acid; *Bacillus mycoides* was found to contain 1.86 per cent phosphorus, 73.65 per cent as nucleic acid. The average phosphorus content of the bacteria examined was found to be 1.5 per cent. Thompson and associates (11) believed that *Az. chroococcum* assimilated more phosphorus during the first 45 days of growth than was made available from tricalcium phosphate but that the reverse was true during the second period of 45 days.

In a study of the biological oxidation of carbohydrates in percolating filters, Jenkins (7) found that the minimum proportion of phosphorus required for the efficient utilization of nitrogen lies between 0.12 and 0.31 parts per part of nitrogen. He showed further that the phosphorus of the organic compounds

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present in the press water of a sugar-beet factor (probably hexophosphate, nucleoproteins, and phospholipins) is not so readily available to the microorganisms in a percolating filter, even in the presence of sufficient available nitrogen, as that of dipotassium phosphate.

The available evidence indicates that phytin, nucleic acid, lecithin, and many other organic phosphorus compounds are excellent sources of phosphorus for a great many microorganisms. The organisms were found to split the phosphorus compounds by means of specific enzymes, giving phosphoric acid, which is readily assimilated. Stoklasa (10) noted that about 66 per cent of the lecithin phosphorus is converted into soluble orthophosphate by *B. mycoides*, *B. subtilis*, and *B. proteus vulgaris* in 60 days at 28–38°C. Bacteria belonging to the genus *Nucleobacter* have been isolated by Koch and Oelsner (8) and found to be specifically active in the decomposition of nucleins, through the nucleic acid stage, into phosphoric acid.

According to Heck (5), the availability and utilization of the organic phosphorus by crops must depend to a large extent upon microbiological activities. Vincent (13) also reported that plants do not assimilate organic phosphorus directly; he attributed the decrease of the organic phosphorus in soil, after crop growth, to its mineralization by bacteria.

EXPERIMENTAL

In the following investigations on the assimilation of phosphorus by a mixed soil population and by pure cultures of soil fungi, cellulose and glucose were used largely as sources of energy.

It was shown in a previous study (1) that phosphorus exerts a pronounced influence upon the rate of decomposition of plant materials. The decomposition of cellulose and hemicelluloses seems to be particularly affected by the added phosphate. The relation of cellulose to the assimilation of nitrogen has been clearly established by Heukelekian and Waksman (6).

Cellulose decomposition by a mixed soil population

Ten-gram portions of finely ground filter paper, equivalent to approximately 9.0 gm. of pure cellulose, and 100-gm. portions of acid-washed white sand were placed in 60 glass tumblers. To 48 of these were added 10-cc. portions of a solution containing 210 mgm. nitrogen as ammonium sulfate, 100 mgm. KCl, 100 mgm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and a trace of ferrous sulfate; the remaining 12 tumblers were treated only with the last three compounds. The tumblers were then divided into groups of 12 each, receiving the following treatments:

- I. No K_2HPO_4
- II. 13.7 mgm. P (0.077 gm. K_2HPO_4)
- III. 36.6 mgm. P (0.206 gm. K_2HPO_4)
- IV. 91.4 mgm. P (0.514 gm. K_2HPO_4)
- V. 94.0 mgm. P (0.528 gm. K_2HPO_4)

After inoculation with 1 cc. of a dilute suspension of fresh field soil, the contents of each tumbler were thoroughly mixed. Water was added at the rate of 10 cc. for each 100 gm. sand and 3 cc. for each gram of paper. The tumblers were covered with glass plates and incubated at 28°C.

Samples were taken after 14, 28, 42, 79, and 121 days. At each period, two tumblers were selected at random from each group. The contents were mixed

TABLE 1

Transformation of phosphorus during the decomposition of cellulose by the mixed soil flora

TREATMENT*	INCUBATION PERIOD	CELLULOSE DECOMPOSED		INORGANIC P FOUND	ORGANIC P	NH ₄ -N FOUND	ORGANIC N	ORGANIC N ORGANIC P	CELLULOSE DECOMPOSED ORGANIC P	CELLULOSE DECOMPOSED ORGANIC N	pH
		gm.	per cent	mgm.	mgm.	mgm.	mgm.				
I. No P	days										
	28	0.472	5.26	0.0	0.0	213.6	-3.6	5.35
	42	0.474	5.28	0.0	0.0	206.6	3.4	4.70
	79	0.331	3.69	0.0	0.0	208.0	2.0
II. 13.7 mgm. P as K ₂ HPO ₄	121	0.148	1.65	0.0	0.0	209.8	0.2
	14	0.523	5.83	10.0	3.7	189.4	20.6	5.57	141.4	25.4
	28	1.587	20.95	8.2	5.5	182.4	27.6	5.02	288.6	57.0	2.80
	42	1.933	21.53	7.2	6.5	175.5	34.5	5.31	297.4	56.0	2.80
III. 36.6 mgm. P as K ₂ HPO ₄	79	2.149	23.94	6.7	7.0	170.5	39.5	5.64	307.7	54.4
	121	2.999	33.40	5.9	7.8	167.6	42.4	5.44	384.4	70.7
	14	0.902	10.05	31.7	4.9	180.8	29.2	5.96	184.1	30.9
	28	2.068	23.03	29.4	7.2	175.0	35.0	4.86	287.0	59.1	2.95
IV. 91.4 mgm. P as K ₂ HPO ₄	42	2.539	29.28	28.1	8.5	170.2	39.8	4.68	298.7	63.8	2.85
	79	2.815	31.35	28.0	8.6	171.5	38.5	4.48	327.4	73.1
	121	4.353	48.49	26.5	10.1	162.7	47.3	4.68	431.0	92.0
	14	1.225	13.64	79.4	12.0	164.5	45.5	3.79	102.1	26.9
V. 94.0 mgm. P as K ₂ HPO ₄ , but no N	28	3.242	36.11	76.7	14.7	158.2	51.8	3.52	220.5	62.6	3.40
	42	3.715	41.38	75.8	15.6	153.4	56.6	3.63	238.1	65.6	3.40
	79	4.372	48.70	75.0	16.4	146.4	63.0	3.84	266.6	69.4
	121	6.185	68.87	73.5	17.9	143.4	66.6	3.72	345.5	92.9
V. 94.0 mgm. P as K ₂ HPO ₄ , but no N	28	0.317	3.53	93.2	0.8	0.0	0.0	7.80
	79	0.062	0.69	94.2	-0.2	0.0	0.0
	121	0.078	0.87	95.3	-1.3	0.0	0.0

* In addition to 100 gm. sand, 8.979 gm. cellulose, minerals, and, except in treatment V, 210 mgm. N as (NH₄)₂SO₄.

and aliquot portions removed for analysis. Phosphorus was estimated colorimetrically by the method of Truog and Meyer (12). Cellulose was determined by hydrolysis with 80 per cent sulfuric acid (14). The results obtained from each aliquot portion were calculated on the basis of the amount of material in each tumbler; only the averages of the duplicates are reported (table 1).

An examination of the organisms concerned in the decomposition of the

cellulose revealed the fact that no cellulose-decomposing bacteria were present. Various other bacteria and the fungus *Chaetomium* sp. were found only in treatments I and V. The absence of bacteria in treatments II, III, and IV is possibly due to the high acidity of the substrate (pH 2.8 to 3.4). The residue from the selective absorption of ammonia from the added ammonium sulfate by cellulose-decomposing organisms was no doubt a major factor contributing to the acidity of the substrate. The active fungus flora consisted of *Trichoderma* sp., of a green *Aspergillus*, and of two *Penicillia*. The first of these increased in growth with the increasing addition of phosphate. During the early stages of decomposition, the *Trichoderma* grew most abundantly in the tumblers receiving the heaviest application of phosphorus. In treatments II and III, the gray *Penicillium* was predominant. The *Penicillium* later appeared also in treatment IV.

It can be noted from table 1 that there had been continuous synthesis of organic phosphorus from the inorganic phosphate during the period of decomposition. This was also true of the nitrogen. The results indicate that there was no accumulation of inorganic compounds of phosphorus or nitrogen from the synthesized organic compounds. Had the period of incubation been sufficiently prolonged, mineralization of the organic compounds might have been expected.

The cellulose was decomposed at a rapid rate in the presence of an abundant supply of phosphate. Whereas there was almost no decomposition in the absence of phosphorus or nitrogen, the decomposition increased steadily to 68.87 per cent where nitrogen and 0.514 gm. of K_2HPO_4 were added. Decomposition was accompanied by synthesis of organic compounds of phosphorus and nitrogen. The greater the amounts of phosphorus added, the narrower were the ratios of organic nitrogen to organic phosphorus, and these ratios remained almost constant within each treatment, irrespective of the period of incubation. The ratio was about 5.4 at the low phosphorus content, 4.9 at the medium phosphorus content, and 3.7 at the high phosphorus content. The amount of cellulose decomposed per unit of nitrogen assimilated averaged 27.4 in 14 days and 60 in 28 days. Higher values were found after longer periods of incubation. The figures are similar to those obtained by Heukelekian and Waksman (6). The presence of phosphorus increased the amount of cellulose decomposed per unit of nitrogen assimilated. The ratios were fairly constant during incubation from 28 to 79 days, but showed further increases to 121 days.

In the presence of moderate amounts of phosphorus, about 160 parts of cellulose were decomposed per unit of organic phosphorus synthesized, in 14 days. This ratio increased to 288 at 28 days. The ratio was lower in the treatment which received the highest amount of phosphate.

It seems reasonable to conclude that the continuous increase in organic phosphorus is due to the fact that there had been an abundant supply of energy-yielding material which greatly stimulated microbial synthesis. This supports

the results reported elsewhere (1), namely, that there is an accumulation of organic phosphorus during decomposition of plant materials rich in cellulose and hemicelluloses.

To observe further the influence of phosphorus upon the decomposition of cellulose, a study on the evolution of CO_2 was made. Two-gram portions of ground filter paper were weighed into 300-cc. aeration flasks containing 100 gm. of acid-washed sand and a solution containing 188.8 mgm. ammonium sulfate, 20 mgm. KCl, 20 mgm. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and a trace of FeCl_3 . These

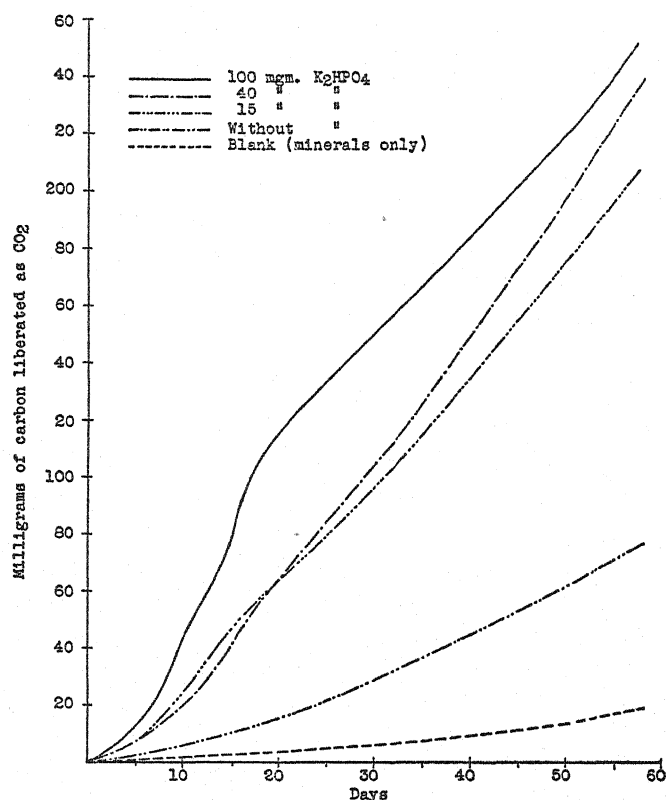


FIG. 1. EFFECT OF PHOSPHORUS UPON THE DECOMPOSITION OF CELLULOSE AS MEASURED BY CO_2 EVOLUTION

flasks were treated, in duplicate, as follows: no P, 15 mgm. K_2HPO_4 , 40 mgm. K_2HPO_4 , 100 mgm. K_2HPO_4 . Two additional flasks containing only sand and nutrients served as checks. The flasks were inoculated with 1 cc. of a dilute suspension (5 per cent) of fresh field soil. Moisture content was made up on the basis of 10 per cent of the dry weight of the sand and of 350 per cent of the dry weight of the filter paper.

After the materials were thoroughly mixed, the flasks were connected to the aeration apparatus and incubated at 28°C . for 59 days. The course of the

evolution of CO_2 is presented graphically in figure 1, and the results of the chemical analyses of the residues are recorded in table 2.

Phosphorus was found to have a pronounced effect upon the decomposition of the cellulose. In the presence of a small amount of available phosphate, cellulose underwent rapid decomposition. There was comparatively little difference in the final amounts of cellulose decomposed with the different applications of phosphate. Transformation was most rapid, however, in the presence of the highest amount of phosphate. In the presence of an abundance of phosphorus, an unusually large amount of this element was assimilated. The ratio between organic nitrogen and organic phosphorus was as low as 1.65:1 with the heaviest phosphate application; all ratios were lower than those previously obtained.

TABLE 2
*Effect of phosphorus upon the decomposition of cellulose**

TREATMENT†	CELLULOSE DECOMPOSED		INOR- GANIC P RECOV- ERED	ORGAN- IC P FORMED	NH ₃ -N RECOV- ERED	ORGAN- IC N FORMED	ORGAN- IC N ORGAN- IC P	pH
	gm.	per cent	mgm.	mgm.	mgm.	mgm.		
Check (minerals only)	7.4	-0.3	35.5	0.1	6.4
No K_2HPO_4	0.095	5.09	35.7	-0.1	4.05
15 mgm. K_2HPO_4 (2.7 mgm. P)	1.074	57.44	trace	2.7	29.5	6.1	2.26	3.65
40 mgm. K_2HPO_4 (7.1 mgm. P)	1.020	54.60	3.8	3.91	24.3	11.3	2.89	3.60
100 mgm. K_2HPO_4 (17.8 mgm. P)	1.069	57.23	11.3	6.5	24.9	10.7	1.65	3.70

* 59 days of incubation.

† In addition to 35.6 mgm. N as $(\text{NH}_4)_2\text{SO}_4$, minerals, and, except in the check, 1.868 gm. cellulose.

Assimilation of phosphorus by soil fungi, with glucose as source of energy

The evidence of extensive synthesis of organic phosphorus during the decomposition of plant materials and cellulose suggested the desirability of making further studies upon the assimilation of phosphorus by a few pure cultures of some of the common soil fungi which are active in the decomposition of organic matter. Particular attention was paid to the effect of different amounts of phosphorus upon the assimilation of phosphorus and upon the utilization of energy-yielding material. The influence of the nitrogen source upon the assimilation of phosphorus was also studied.

The following fungi were used: *Asp. niger*, *Rhizopus nigricans*, a *Trichoderma*, a gray *Penicillium*, and an *Aspergillus*. The last three fungi were isolated from the experiments on cellulose decomposition.

A basic medium of the following composition was adopted:

Glucose.....	20 gm.
KCl.....	0.200 gm.
MgSO ₄ ·7H ₂ O.....	0.200 gm.
FeSO ₄	0.005 gm.
ZnSO ₄	0.001 gm.
Distilled water.....	1000 cc.

The basic medium, in 200-cc. portions, was measured into 500-cc. Erlenmeyer flasks. The flasks were divided into two sets, one of which received 0.5 per cent concentration of K₂HPO₄ solution, and the other, 1.5 per cent. After sterilization at 10 pounds pressure for 30 minutes, ammonium sulfate, sodium nitrate, and urea were supplied as sources of nitrogen for *Asp. niger*, whereas only urea was supplied to the other fungi. Solutions of the nitrogen compounds were prepared in such concentrations that each 10 cc. gave 114.5 mgm. of nitrogen per flask. These solutions were sterilized separately and 10-cc. portions added to the flasks. There were 9 to 12 replicates of each medium for each fungus. Uninoculated controls were also included. The amounts of the nitrogen, phosphorus, and glucose in the control flasks were considered to have been the original amounts in all the flasks. The materials were inoculated with spore suspensions of the fungi and incubated at 28°C.

Three flasks from each treatment, inoculated with each fungus, were taken for analysis at weekly intervals. The solution was drained through weighed filter paper into 500-cc. volumetric flasks. The fungus pellicle remaining in the Erlenmeyer flask was washed three times with a small amount of 1 per cent HCl solution and then washed free of acid, upon the filter paper. The paper containing the pellicle was dried for 10 to 12 hours at 80°C. and then at 100°C. to constant weight. The weight of the paper with the pellicle minus that of the original paper gave the weight of the fungus mycelium produced during incubation. The spent medium and the washings were made up to 500 cc.; aliquot portions were withdrawn for determinations of inorganic phosphate, nitrogen, and glucose. Inorganic phosphate was estimated by the colorimetric method of Truog and Meyer; glucose by Bertrand's method; nitrate by reduction with Devarda's alloy; ammonia by distillation with MgO. To estimate urea, 50 cc. of the filtrate was neutralized and treated for 1 hour with 3 cc. of an extract of 10 per cent Jack bean powder in 30 per cent alcohol. The ammonia was distilled in the presence of MgO.

Phosphorus assimilation by Asp. niger. It was apparent, even on general examination, that the growth of *Asp. niger* was heavier in the presence of ammonium sulfate than with urea or with nitrate, irrespective of the concentration of phosphate. The pellicles in the ammonium sulfate medium were heavy and much folded, whereas in the nitrate medium they were thin and light in color. The color was usually lighter with higher phosphate concentrations.

Several interesting points are brought out in tables 3, 4, and 5. The greater amount of phosphate depressed the growth of the fungus but stimulated the

TABLE 3
Assimilation of phosphorus by Asp. niger in medium with (NH₄)₂SO₄

TREATMENT*	AGE OF CULTURE	MYCELIUM†	ORGAN-IC P SYNTHESIZED	ORGAN-IC P, PER CENT OF MYCELIUM	N ASSIMILATED	N, PER CENT OF MYCELIUM	N ASSIMILATED ORGAN-IC P	GLUCOSE CONSUMED	pH‡
	days	gm.	mgm.		mgm.			gm.	
0.0969 gm. K ₂ HPO ₄ (0.48 per cent)	7	0.817	9.4	1.15	54.0	6.61	5.74	2.332	2.57
	14	1.151	13.1	1.14	69.7	6.06	5.32	3.399	2.23
	21	1.057	11.3	1.07	63.0	5.96	5.58	3.474	2.43
	28	0.968	7.6	0.79	56.8	5.87	7.47	2.33
0.3057 gm. K ₂ HPO ₄ (1.53 per cent)	7	0.757	6.7	0.89	49.4	6.53	7.37	2.192	2.55
	14	1.021	14.5	1.42	63.3	6.20	4.37	3.077	2.32
	21	0.988	12.7	1.29	60.2	6.09	4.74	3.474	2.53
	28	0.897	8.1	0.90	52.0	5.80	6.42	2.53

* In addition to the basic medium (including 3.474 gm. glucose), 114.5 mgm. N, etc.

† Oven-dry weight.

‡ The original pH of 0.48 per cent phosphate solution was 6.1, and that of 1.53 per cent phosphate solution was 6.45.

TABLE 4
Assimilation of phosphorus by Asp. niger in medium with NaNO₃

TREATMENT*	AGE OF CULTURE	MYCELIUM†	ORGAN-IC P SYNTHESIZED	ORGAN-IC P, PER CENT OF MYCELIUM	N ASSIMILATED	N, PER CENT OF MYCELIUM	N ASSIMILATED ORGAN-IC P	GLUCOSE CONSUMED	pH‡
	days	gm.	mgm.		mgm.			gm.	
0.0969 gm. K ₂ HPO ₄ (0.48 per cent)	7	0.493	6.3	1.28	29.4	5.96	4.67	2.193	2.77
	14	0.731	8.1	1.11	43.5	5.95	5.37	2.949	2.72
	21	0.767	9.3	1.12	47.5	6.19	5.11	3.474	3.02
	28	0.735	7.5	1.02	45.5	6.19	6.07	3.18
0.3057 gm. K ₂ HPO ₄ (1.53 per cent)	7	0.413	4.0	0.97	27.0	6.54	6.75	2.080	3.23
	14	0.578	7.5	1.30	36.8	6.37	4.91	2.949	2.89
	21	0.596	10.3	1.73	38.5	6.46	3.74	3.474	2.57
	28	0.548	7.6	1.39	35.9	6.55	4.72	2.93

* In addition to the basic medium (including 3.474 gm. glucose), 114.5 mgm. N, etc.

† Oven-dry weight.

‡ The original pH of 0.48 per cent phosphate solution was 6.5, and that of 1.53 per cent phosphate solution was 6.70.

utilization of glucose per unit of mycelium produced. The ratio of organic nitrogen to organic phosphorus was also narrower at the high concentration of phosphorus. The fungus mycelium contained, on the average, 6 per cent or-

ganic nitrogen and 1.1 per cent phosphorus at low phosphate concentration and 1.3 per cent phosphorus at high concentration.

The greatest increase in weight of mycelium and synthesis of organic phosphorus took place in 14 days in the media with ammonium sulfate and urea

TABLE 5
Assimilation of phosphorus by Asp. niger in medium with urea

TREATMENT*	AGE OF CULTURE	MYCELIUM†	ORGANIC P SYNTHESIZED	ORGANIC P, PER CENT OF MYCELIUM	N ASSIMILATED	N, PER CENT OF MYCELIUM	N ASSIMILATED ORGANIC P	GLUCOSE CONSUMED	pH‡
	days	gm.	mgm.		mgm.			gm.	
0.0969 gm. K ₂ HPO ₄ (0.48 per cent)	7	0.677	7.5	1.11	41.3	6.10	5.51	2.248	2.71
	14	0.939	10.1	1.08	52.9	5.63	5.24	3.158	2.83
	21	0.915	9.5	1.04	55.8	6.10	5.87	3.474	3.28
	28	0.893	7.5	0.84	53.8	6.02	7.17	3.60
0.3057 gm. K ₂ HPO ₄ (1.53 per cent)	7	0.430	5.5	1.28	41.3	9.60	7.51	1.975	3.27
	14	0.740	11.7	1.58	42.1	5.69	3.60	2.916	2.87
	21	0.627	10.1	1.61	42.4	6.77	4.20	3.474	3.00
	28	0.677	8.31	1.23	45.0	6.64	5.42	3.13

* In addition to the basic medium (including 3.474 gm. glucose), 114.5 mgm. N, etc.

† Oven-dry weight.

‡ The original pH of 0.48 per cent phosphate solution was 7.05, and that of 1.53 per cent phosphate solution was 6.40.

TABLE 6
Assimilation of phosphorus by R. nigricans in medium with urea

TREATMENT*	AGE OF CULTURE	MYCELIUM†	ORGANIC P SYNTHESIZED	ORGANIC P, PER CENT OF MYCELIUM	N ASSIMILATED	N, PER CENT OF MYCELIUM	N ASSIMILATED ORGANIC P	GLUCOSE CONSUMED
	days	mgm.	mgm.		mgm.			gm.
0.1012 gm. K ₂ HPO ₄	7	637.3	10.2	1.60	42.4	6.65	4.16	2.683
	14	881.0	13.1	1.49	57.8	6.56	4.41	3.229
	21	835.3	13.9	1.66	54.3	6.50	3.91	3.263
0.3046 gm. K ₂ HPO ₄	7	502.3	16.8	3.34	34.6	6.89	2.06	2.351
	14	667.3	15.2	2.28	47.9	7.18	3.15	3.154
	21	670.0	19.9	2.97	41.7	6.22	2.10	3.188

* In addition to the basic medium (including 3.263 gm. glucose), 114.5 mgm. of N in the form of urea, etc.

† Oven-dry weight.

and in 21 days in the nitrate medium. After the glucose had all disappeared, there was some autolysis of the mycelium, as shown by the decreased weight of the mycelium, and by the lower amounts of organic phosphorus and organic nitrogen. Autolysis was much delayed in the nitrate medium. The organic

phosphorus decreased more rapidly than the organic nitrogen, resulting in a widening of the ratio of organic nitrogen to organic phosphorus with prolonged incubation.

TABLE 7
Assimilation of phosphorus by Trichoderma sp. in medium with urea

TREATMENT*	AGE OF CULTURE	MYCELIUM†	ORGANIC P SYNTHESIZED	ORGANIC P, PER CENT OF MYCELIUM	N AS-SIMILATED	N, PER CENT OF MYCELIUM	N AS-SIMILATED ORGANIC P	GLUCOSE CONSUMED
	days	mgm.	mgm.		mgm.			gm.
0.1012 gm. K_2HPO_4	7	595.3	6.5	1.09	43.2	7.26	6.65	1.285
	14	806.0	13.4	1.66	58.0	7.20	4.33	2.838
	21	697.8	8.3	1.71	49.1	7.04	5.92	3.164
0.3046 gm. K_2HPO_4	7	508.8	7.3	1.43	33.4	6.56	4.58	1.128
	14	904.0	31.5	3.48	61.4	6.79	1.95	2.801
	21	805.5	14.1	1.75	57.4	7.13	4.07	3.137

* In addition to the basic medium (including 3.263 gm. glucose), 114.5 mgm. of N in the form of urea, etc.

† Oven-dry weight.

TABLE 8
Assimilation of phosphorus by Aspergillus sp. and Penicillium sp. in medium with urea

TREATMENT*	AGE OF CULTURE	MYCELIUM†	ORGANIC P SYNTHESIZED	ORGANIC P, PER CENT OF MYCELIUM	N AS-SIMILATED	N, PER CENT OF MYCELIUM	N AS-SIMILATED ORGANIC P	GLUCOSE CONSUMED
<i>Aspergillus sp.</i>								
	days	mgm.	mgm.		mgm.			gm.
0.1012 gm. K_2HPO_4	7	405.8	3.4	0.84	17.2	4.24	5.06	0.686
	14	856.8	13.6	1.59	44.3	5.17	3.26	2.217
0.3046 gm. K_2HPO_4	7	461.0	7.7	1.67	23.6	5.12	3.06	1.043
	14	868.0	36.1	4.16	46.0	5.30	1.27	2.552
<i>Penicillium sp.</i>								
0.1012 gm. K_2HPO_4	14	418.0	11.7	2.78	20.0	4.78	1.71	1.282
	21	752.0	4.8	0.53	38.8	5.16	8.08	2.413
0.3046 gm. K_2HPO_4	14	506.5	33.4	6.59	30.2	5.96	0.90	1.719
	21	718.0	8.2	1.14	43.9	6.11	5.35	2.738

* In addition to the basic medium (including 3.263 gm. glucose), 114.5 mgm. of N in the form of urea, etc.

† Oven-dry weight.

Phosphorus assimilation by R. nigricans, Trichoderma sp., Aspergillus sp., and Penicillium sp. In the glucose-urea medium, *Trichoderma sp.*, *Aspergillus sp.*, and *Penicillium sp.* produced only submerged growth. Development

SURVIVAL OF MICROORGANISMS INOCULATED INTO STERILIZED SOIL¹

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The subject of antagonistic reactions among microorganisms has received considerable attention in recent years (5, 13). In view of its possible practical significance in regard to biological control of plant parasites, it deserves further careful consideration. Various combinations of organisms have been tested on artificial media, but the results obtained were not always reproducible in more complex substrates such as soil (8). Sterilized or partially sterilized soils have been used by many investigators in studies of antagonism among microorganisms, despite the recognizedly abnormal conditions in soils subjected to heat or chemical treatment (12).

Conn and Bright (3) studied the behavior of *Bacillus cereus*, *Pseudomonas fluorescens*, and *Bacterium caudatus* individually and together in sterilized manured soil and found that the last two organisms increased rapidly in numbers but that *B. cereus* multiplied more slowly. When the three organisms were inoculated simultaneously into the same medium, *B. cereus* was completely inhibited, whereas the other two forms multiplied rapidly. Millard and Taylor (9) inoculated *Actinomyces scabies* and *Act. praecox* into partially sterilized soils and found a marked reduction of potato scab. In other experiments it was found that the numbers of *Act. scabies* decreased to zero in most cases when in combination with *Act. praecox*. Lewis (8) tested various fungi, bacteria, and actinomycetes against *Ps. fluorescens* in artificial media and found that, as a rule, the actinomycetes were more sensitive than the fungi but that certain spore-forming bacteria (*B. mycoides*) were inhibited completely. When inoculated into sterile soils in which *Ps. fluorescens* had grown for 14 days, *B. cereus* was not inhibited. Lewis concluded that "the theory that toxic metabolic products exert the same effect in soils as in artificial media is not supported." Allen and Haenseler (1), using soils sterilized with formaldehyde, obtained evidence that the combination of *Trichoderma lignorum* with *Rhizoctonia* and *Pythium* resulted in decreased infection of cucumber and pea seeds. The previous observations of Weindling (13) were thus confirmed. In sterilized soil, combination with pure cultures of certain soil bacteria was not deleterious to avian tubercle bacilli, which actually multiplied (11); however,

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

a certain fungus checked development of the tubercle bacillus in manured sterilized soil, and the bacillus was destroyed slowly in nonsterilized soils. *Trichoderma lignorum* and several other fungi when introduced into sterilized soils artificially inoculated with *Helminthosporium sativum* suppressed seedling injury (2). Novogrudsky (10) inoculated sterilized soil with a bacterium causing lysis of *Fusarium lini*; 24 hours later he introduced the pathogenic fungus, which proved to be ineffective in the presence of the bacterium; the wheat seedlings used as indexes of infection were thus completely protected.

EXPERIMENTAL

Survival of various organisms inoculated into soil

Palouse silt loam, in 100-gm. portions, was placed in 250-cc. Erlenmeyer flasks and sterilized for 2.5 hours at 15 pounds pressure. Suspensions of the various organisms were made from young agar cultures by the use of sterile distilled water; spore suspensions were used for fungi, and a mixture of mycelium and spores was used for actinomycetes. A quantity of each suspension sufficient to bring the moisture of the soil up to 50 per cent of its moisture-holding capacity was added aseptically to each flask. The soils were kept undisturbed for 24 hours to permit the uniform distribution of moisture and organisms. Samples were then removed aseptically and plated with nutrient and sodium-albuminate agars for bacteria and actinomycetes and with peptone-acid agar for fungi (4).

The organisms used in these experiments were obtained from the culture collection of the soils department at the New Jersey Agricultural Experiment Station. The results obtained in the first experiment are presented in table 1. The numbers of each organism, with the exception of *Azotobacter*, increased to a maximum, which varied with the particular organism, and then gradually decreased. The increase may be attributed to two factors; namely, an increase in available nutrients as a result of sterilization (12), and the absence of competitive and antagonistic organisms. The inability of *Az. chroococcum* to multiply in sterilized soil could possibly be explained by a lack of suitable nutrients (7) or by the production of toxic materials during sterilization.

Survival of combinations of different organisms inoculated into soil

Combinations of various organisms were next used in an attempt to demonstrate any antagonistic effects. Species of fungi, bacteria, and actinomycetes which produced readily recognizable, characteristic colonies on plates were used. The organisms were introduced into 100-gm. quantities of sterile Palouse soil placed in 250-cc. flasks.

Certain representative results are reported in tables 2 and 3. *Cunninghamella* in combination with *Humicola* reached its numerical peak much sooner and decreased more rapidly than when alone; *Humicola* was not particularly affected by the association. *Act. cellulosa* was repressed by *Act. violaceus ruber*, since at the maximum, its numbers were three times the original, whereas when alone it multiplied to a peak approximately ten times as great as the

numbers at the beginning. Similarly, it may be observed that *B. megatherium* in conjunction with *Az. chroococcum* and *Ps. fluorescens* did not multiply appreciably, whereas in pure culture its numbers increased in 14 days to about twenty times the original. *Azotobacter* also decreased more rapidly in combination than when alone; *Ps. fluorescens* was not noticeably affected.

TABLE 1

Survival of pure cultures of organisms inoculated singly into sterilized soil
Numbers per gram of oven-dry soil, $\times 10^{-3}$

ORGANISM	DAYS OF INCUBATION				
	0	3	14	40	80
<i>Act. violaceus ruber</i>	72	563	2,619	12,234	8,234
<i>Act. cellulosa</i>	2,824	1,085	9,213	29,736	19,987
<i>Act. fradrii</i> 3322b.....	984	6,532	7,217	19,932	9,634
<i>Az. chroococcum</i> *.....	2,200	1,050	1,490	1,320	990
<i>Ps. fluorescens</i> †.....	1,824	2,011	3,068	3,792	2,782
<i>B. cereus</i>	4	12	19	18	14
<i>B. megatherium</i>	2	10	37	18	13
<i>B. mycoides</i>	1	6	9	11	8
<i>Humicola</i> sp.....	59	288	651	934	659
<i>Aspergillus</i> sp.....	419	2,324	2,700	4,129	3,736
<i>Trichoderma</i> sp.....	344	1,542	1,721	2,039	2,432
<i>Penicillium</i> sp.....	1,170	6,418	14,304	19,267	20,692
<i>Cunninghamella</i> sp.....	60	337	430	601	501

* Numbers of *Azotobacter* per gram oven-dry soil.

† *Ps. fluorescens* $\times 10^{-5}$ per gram oven-dry soil.

TABLE 2

Survival of various combinations of organisms inoculated into sterilized soil
Numbers per gram oven-dry soil, $\times 10^{-4}$

INCUBATION	CUNNINGHAMELLA + HUMICOLA	ACT. VIOLA-CEUS RUBER + ACT. CELLULOSAE	AZ. CHROCOCCUM* + PS. FLUORESCENS + B. MEGATHERIUM				
<i>days</i>							
0	0.2	0.3	4	132	2,000	2,175	818
3	10	0.8	2	146	2,090	2,782	841
10	65	6	88	167	1,760	15,227	568
24	33	99	217	373	1,000	16,361	775
50	30	27	893	373	530	8,765	970
80	19	8	1,194	267	220	9,398	897

* *Azotobacter* per gram oven-dry soil.

In the mixture of four organisms, *Humicola* and *Act. violaceus ruber* were not materially affected by their association. *Act. cellulosa* again was somewhat repressed, whereas *Cunninghamella* was slightly stimulated as compared to its multiplication in pure culture. *Azotobacter* again decreased more rapidly in the presence of other organisms than when alone. In the latter case nearly half of the cells were still alive after 80 days, whereas in the former only

one-fifth of the original numbers survived. *Ps. fluorescens* and *Cunninghamella* multiplied more extensively together than when in pure culture, *Humicola* was not particularly affected, and *B. megatherium* was somewhat inhibited after a rapid increase during the first few days of incubation.

Development of plant pathogenic fungi in soil

Several plant pathogenic fungi (6) were inoculated into sterile soil individually and with other fungi, bacteria, and actinomycetes (tables 4, 5). As difficulty

TABLE 3
Survival of various combinations of organisms inoculated into sterilized soil
Numbers per gram oven-dry soil, $\times 10^{-4}$

INCUBATION	CUNNINGHAMELLA +	HUMICOLA +	ACT. VIOLA-CEUS RUBER +	ACT. CELLULOSAE	AZ. CHROCOCCUM* +	PS. FLUORESCENS +	B. MEGATHERIUM +	CUNNINGHAMELLA +	HUMICOLA
days									
0	1	1	4	67	1,300	2,610	178	1	0.4
3	13	1	5	75	400	16,820	3,830	6	2
10	13	2	53	71	250	18,360	1,010	11	2
24	31	26	309	112	360	13,650	144	151	12
50	41	29	734	216	220	9,753	103	199	19
80	20	10	934	154	260	10,010	89	23	24

* *Azotobacter* per gram oven-dry soil.

TABLE 4
Persistence of some plant pathogenic fungi alone and in combination with various organisms in sterilized soil
Numbers per gram oven-dry soil, $\times 10^{-4}$

INCUBATION	RHIZOCTONIA SOLANI	HELMINTHOSPORIUM SATIVUM	FUSARIUM CULMORUM	CUNNINGHAMELLA +	ASPERGILLUS	F. CULMORUM +	CUNNINGHAMELLA +	ASPERGILLUS
days								
0	0.09	0.07	75	2	32	16	9	38
4	0.14	1.36	79	18	47	37	18	21
14	0.22	3.34	205	39	66	58	33	18
31	0.45	1.66	335	26	58	69	25	8
43	0.37	0.93	114	24	54	39	29	6
61	0.26	0.31	109	21	55	24	32	5

was experienced in counting *Rh. solani* and *H. sativum* in the presence of such rapidly spreading fungi as *Cunninghamella* and *Aspergillus*, only *F. culmorum* was used in combination with these two organisms.

All three pathogens grew when inoculated into sterile soil. *Cunninghamella* developed normally in association with *Aspergillus*, but the latter was somewhat repressed as compared to its development in pure culture. In combination with *F. culmorum* and *Cunninghamella*, *Aspergillus* was distinctly inhibited. *Rhizoctonia solani* and *Act. cellulosa* were both repressed in the presence of *Act. fradii* (table 5). *Act. cellulosa* was also inhibited, when together with

Helminthosporium and *Act. fradii*; the fungus was also affected, since when alone its increase to a maximum was about 40-fold, whereas in combination it increased to about 10 times the original. Combination with *Rhizoctonia* was more deleterious to *Act. cellulosa* than association with *Helminthosporium* or *F. culmorum*. The coexistence of two bacteria with *Rh. solani* resulted in a strong inhibition of the fungus; *Ps. fluorescens* increased gradually, as did *B. cereus*. The combination of *H. sativum* with the two bacteria was not the most favorable for any of the three; the fungus developed to a maximum of ten

TABLE 5

Persistence of some plant pathogenic fungi alone and in combination with various organisms in sterilized soil

Numbers per gram oven-dry soil, $\times 10^{-4}$

INCUBATION	R. SOLANI	ACT. CELLULOSAE	ACT. FRADII 3322b	H. SATIVUM	ACT. CELLULOSAE	ACT. FRADII 3322b
days						
0	0.069	349	4	0.13	719	2.8
4	0.060	308	5	0.86	649	5.3
14	0.059	262	17	1.55	594	8.8
31	0.047	155	69	0.91	1,617	20.0
43	0.043	104	..	0.72	1,513
61	0.031	89	..	0.37	1,379
	F. CULMORUM	ACT. CELLULOSAE	ACT. FRADII 3322b	R. SOLANI	PS. FLUORESCENS	B. CEREUS
0	3.5	304	12	0.120	2,800	186
4	17.6	316	9	0.080	5,370	286
14	40.0	447	4	0.040	7,470	462
31	59.0	1,062	11	0.020	8,540	532
43	57.0	973	..	0.010	9,730	432
61	59.0	728	..	0.009	15,750	362
	H. SATIVUM	PS. FLUORESCENS	B. CEREUS	F. CULMORUM	PS. FLUORESCENS	B. CEREUS
0	0.27	19,330	512	56	6,850	194
4	0.56	9,420	552	96	3,210	174
14	1.03	2,630	672	143	2,264	191
31	3.03	577	577	167	2,720	256
43	0.97	836	503	123	7,320	194
61	0.34	11,850	415	90	15,930	108

times the initial inoculum, whereas in pure culture it increased 40-fold. *B. cereus* did not increase perceptibly and *Ps. fluorescens* actually decreased during the first 43 days. A similar repression of *B. cereus* was noted in combination with *Ps. fluorescens* and *F. culmorum*; the latter two organisms were not appreciably affected.

DISCUSSION

Only the most conservative conclusions can be drawn from the data presented on the coexistence of different organisms in sterilized soil, especially

since the same numbers of cells were not always present initially in the different combinations used. This factor is difficult to control, and considerable variation is to be expected. Even the introduction of the same quantity of inoculum into two 100-gm. samples of one soil does not yield similar numbers when both samples are plated out; this was also found to be the case when two portions of the same soil were tested. Consequently, conclusions may be drawn only when a particular result appears consistently. *B. megatherium* and *Act. cellulosa*, for example, were most often repressed when in association with other microorganisms.

Caution should also be exercised in interpreting the results of fungus counts in soil, since there are no means of determining how many of the colonies developing on the plates are due to spores and how many to pieces of mycelium. A certain treatment may inhibit sporulation yet increase mycelial development; thus the number of colonies on the plates may be reduced (assuming that they originated from spores), yet the organism may be very active in the substrate. Similar objections may be raised also to counts of actinomycetes in soil.

The data presented support the work of many investigators in demonstrating that certain organisms can develop in sterilized soils as a result of several favorable factors brought about by sterilization, namely, the absence of an active competing microflora and the increase of available nutrients because of the steam treatment. These two factors may be complementary, since the presence of an active microbial population may result in a lack of available nutrients for the inoculants. It appears that antagonistic phenomena also occurred in the complex soil system. The responses were often not distinct, but in some cases there was a consistent inhibition of several organisms. Considerable variation is expected in work with the heterogeneous soil substrate.

The importance of isolating the biological systems to be studied (by use of sterile substrates) before experimenting with the reactions of inoculants to the complex biological communities in the soil appears obvious, as is the case also with the use of a complex substrate such as soil. The conclusion of Lewis (8) that the theory that toxic metabolic products exert the same effect in soils as in artificial media was not supported by his results is entirely reasonable because of absorption and other phenomena in soils which tend to inactivate such toxic materials. Consequently, it might be expected that any inhibitory effects which can be demonstrated clearly in soil are the result of pronounced antagonistic reactions. The problem still exists, however, as to whether similar inhibition will occur in nonsterilized soil.

SUMMARY

Some typical soil fungi, bacteria, and actinomycetes were inoculated singly and in combination into steam-sterilized soil. All the organisms, with the exception of *Azotobacter chroococcum* increased in numbers to a maximum and then gradually decreased; the number of *Azotobacter* decreased steadily.

NUTRITION STUDIES WITH CORN: III. A STATISTICAL INTERPRETATION OF THE RELATION BETWEEN NUTRIENT ION CONCENTRATION AND THE CARBOHYDRATE AND NITROGENOUS CONTENT OF THE TISSUE¹

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A review of the voluminous literature on the effects of the various nutrient ions in the substrate upon carbohydrate-nitrogen relationships brings to light such extreme contradictions in conclusions that a new approach to this relation was sought. Many studies have been based on variations in concentration of one salt from complete deficiency to definite excess, in the presence of constant concentrations of other salts. If, for example, the cation to be studied were K^+ , it might be introduced with an anion such as Cl^- , which was then considered to have little or no physiological significance. This manner of manipulation is not entirely satisfactory, inasmuch as it involves changes in substrate concentration, the effects of which cannot be adequately evaluated in a study of the comparative effects of the nutrient ions upon metabolic relationships. Both of the ions resulting from the addition of a salt for the purpose principally of varying the concentration of one ion are capable of satisfying electrical charges within the tissue and therefore must necessarily have some physiological significance. These ions enter into the competitive interionic relationships discussed in a previous paper (3).

The present approach to this problem was through the use of the variable ion proportion series of solutions described in previous reports of this series (2, 3).

THE EXPERIMENTAL SYSTEM

Inasmuch as this is the third in a series of papers all of which are based upon the same experimental system, the reader is referred to the first paper for a detailed description of the procedure involved (3).

Very briefly stated, a series of 16 solutions were devised, using nitrates of the three nutrient cations K^+ , Ca^{++} , and Mg^{++} and the sulfates and phosphates of the same three cations in a manner such that the concentration of each of these ions could be varied. All nutrient solutions have an osmotic concentration of approximately 0.5 atmosphere. Boron, iron, and manganese were each

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

added at a concentration of 0.5 p.p.m. of the culture solution. Triplicate cultures of four plants each of Dave Croshaw's strain of Reid's yellow dent corn were grown with each of the nutrient solutions of this series. The plants were harvested after an experimental period of 40 days; fresh and dry weight records were taken, and suitable aliquots of leaf and stem tissue were put in glass-topped fruit jars and quickly frozen by means of dry ice.

All data are calculated in terms of milligrams per 100 gm. fresh tissue.

ANALYTICAL METHODS

The frozen tissue was ground thoroughly through an ordinary food chopper, care being taken to prevent loss of juice, and was then extracted with hot water, according to the procedure of Nightingale, Robbins, and Schermerhorn (8), acetic acid being used as the protein precipitant prior to filtration (4). Aliquots of the filtered extract were used in making all the soluble nitrogen determinations.

Ammonium and nitrate nitrogen were determined by the aspiration method of Sessions and Shive (11).

Alpha-amino nitrogen was determined by the Van Slyke procedure (7), the filtrate from the basic nitrogen determination being used.

Amide nitrogen was determined by hydrolyzing duplicate aliquots with 5 per cent H_2SO_4 for $2\frac{1}{2}$ hours and determining the increase in ammonium nitrogen by aspiration.

Basic nitrogen was determined by precipitation with phosphotungstic acid and by determining the total nitrogen of the precipitate (9).

Total soluble nitrogen was determined by the use of Ranker's modification (10) of the Kjeldahl procedure.

Total nitrogen was determined from tissue dried at 75°C . and finely ground in a ball mill. Ranker's (10) Kjeldahl method was again used.

Protein nitrogen was calculated as the difference between total nitrogen and total soluble nitrogen.

Soluble organic nitrogen, total organic nitrogen, and other nitrogen (soluble), are calculated values of obvious derivation.

The carbohydrate fractions were determined from tissue quickly dried at 75°C . and pulverized in a ball mill so that it all passed through a 100-mesh sieve.

Reducing sugars were determined, by the Tompsett procedure (12), from the filtrate obtained after refluxing the tissue for one-half hour with 85 per cent ethyl alcohol. For the standardization of the thiosulfate used in the final titration, c. p. glucose was used.

Sucrose was determined as the difference between the glucose equivalent of the thiosulfate titrations of aliquots of the filtrate after deleading in the Tompsett procedure (12) before and after inversion with invertase.

Starch and dextrins were determined from the residue of the reducing sugar filtration. The residue was refluxed with water for one-half hour, cooled at

35°C., and digested with fresh saliva for 1 hour. After filtration, the glucose equivalent was determined.

Hemicelluloses were determined from the residue of the starch filtration by refluxing for 2½ hours in a solution containing 2.5 per cent HCl. The glucose equivalent was determined in the filtrate after clearing and deleading.

PRESENTATION OF DATA

Tables 1 and 2 present the quantities of the various nitrogen fractions determined in the stem and the leaf tissue respectively. A comparison of these tables with tables 3 and 4 gives some evidence of the reliability of the statistical consideration of these data. As total nitrogen and total soluble

TABLE 1

Nitrogen fraction distribution in corn stem tissue, expressed in milligrams of nitrogen per 100 gm. of fresh tissue

TREAT- MENT NUMBER	NH ₄ -N	NO ₂ -N	AMIDE N	AMINO N	BASIC N	TOTAL SOLUBLE N	SOLUBLE ORGANIC N	OTHER N (SOL- UBLE)	PROTEIN N	TOTAL ORGANIC N	TOTAL N
1	0.85	25.7	1.05	11.4	0.76	50.4	23.8	10.7	41.5	65.4	91.8
2	1.19	7.5	1.31	2.3	1.17	27.8	19.1	14.4	44.4	63.6	72.3
3	1.53	101.1	2.56	17.1	0.91	132.0	28.9	8.4	40.0	69.0	171.8
4	0.63	8.8	1.36	3.2	1.10	29.4	19.8	14.2	36.2	56.0	65.6
5	3.07	87.4	3.74	20.7	0.82	126.0	35.9	10.0	50.7	86.5	177.0
6	0.68	30.3	4.08	8.3	0.86	58.0	27.0	14.1	56.4	83.4	114.2
7	1.36	4.3	1.34	7.5	0.66	30.0	24.4	14.9	47.5	71.9	77.6
8	1.07	5.3	2.32	7.3	0.71	29.0	22.6	12.3	63.8	86.5	92.8
9	0.85	6.2	1.71	4.5	1.04	25.2	18.1	10.9	42.8	60.9	68.0
10	0.85	16.6	1.06	7.5	0.88	37.6	20.2	10.8	40.5	60.6	78.1
11	2.39	69.4	0.55	7.8	1.23	91.6	19.8	10.3	49.7	69.5	141.0
12	0.68	5.5	1.58	4.5	1.03	26.4	20.3	13.3	43.6	63.9	70.2
13	1.28	10.9	0.79	10.0	0.66	39.5	27.2	15.9	57.2	84.6	96.8
14	1.70	89.1	4.89	18.7	1.41	123.0	32.4	7.9	60.6	93.0	183.8
15	1.70	120.8	5.80	21.2	1.63	158.2	35.5	6.9	43.2	78.7	201.0
16	0.85	4.9	2.26	8.7	0.67	38.6	29.0	17.4	50.5	79.5	85.3

nitrogen were considered statistically in the preceding paper (2), they will not be considered further. The "other nitrogen" fraction will not be considered statistically because of its undetermined composition and also because variations of this fraction are, in general, similar to variations of the other organic nitrogen fractions.

The data concerning the carbohydrate fractions in the stem and the leaf tissue, presented in tables 5 and 6 respectively, include the residuals from the statistical analysis.

STATISTICAL CONSIDERATION OF DATA

The statistical methods employed in the analysis (6) of data were substantially the same as those used in the previous papers of this series. The

TABLE 2

Nitrogen fraction distribution in corn leaf tissue, expressed in milligrams of nitrogen per 100 gm. of fresh tissue

TREAT- MENT NUMBER	NH ₄ -N	NO ₃ -N	AMIDE N	AMINO N	BASIC N	TOTAL SOLUBLE N	SOLUBLE ORGANIC N	OTHER N (SOL- UBLE)	PROTEIN N	TOTAL ORGANIC N	TOTAL N
1	1.02	7.8	5.12	24.1	1.69	75.3	66.5	36.6	248.0	315.0	324.0
2	1.33	3.8	1.71	16.9	1.19	57.3	52.2	33.6	204.0	256.0	261.0
3	2.37	48.1	3.84	28.2	1.92	135.6	85.2	53.5	306.0	390.0	441.0
4	1.70	3.4	1.71	10.6	1.43	44.8	39.7	27.6	208.0	247.0	252.0
5	2.38	60.2	6.82	45.9	2.28	142.2	80.0	27.4	328.0	408.0	470.0
6	2.73	18.7	3.84	33.2	1.89	88.9	67.4	31.2	310.0	378.0	399.0
7	2.37	2.7	3.84	19.4	1.52	57.7	52.6	30.1	219.0	271.0	276.0
8	2.04	5.1	2.55	23.6	1.52	51.6	44.5	18.8	235.0	279.0	286.0
9	0.68	4.8	2.13	13.0	1.46	45.5	40.0	24.1	198.0	238.0	244.0
10	1.70	9.2	3.40	19.8	1.99	64.5	53.7	30.1	267.0	320.0	332.0
11	1.02	23.2	3.84	21.4	2.17	92.3	68.0	41.6	261.0	330.0	354.0
12	2.37	6.1	2.97	16.5	1.42	50.7	42.3	23.7	206.0	248.0	257.0
13	1.37	4.1	2.56	17.7	1.43	62.9	57.4	36.9	228.0	285.0	291.0
14	4.41	69.4	7.64	45.3	1.87	155.5	81.8	31.4	263.0	345.0	418.0
15	3.05	48.1	5.97	46.3	2.20	160.0	102.6	51.0	282.0	384.0	435.0
16	1.71	6.8	3.41	26.3	1.13	61.6	53.1	23.9	239.0	292.0	300.0

TABLE 3

Z' values, or residuals of the statistical analysis of the nitrogen fractions in corn stem tissue

TREAT- MENT NUMBER	NH ₄ -N	NO ₃ -N	AMIDE N	AMINO N	BASIC N	SOLUBLE ORGANIC N	PROTEIN N	TOTAL ORGANIC N
1	0.07	-13.4	-1.02	1.5	-0.23	0.3	-0.8	-0.4
2	0.44	2.9	0.12	-1.8	0.26	-0.7	1.5	0.6
3	-0.40	7.8	0.09	1.9	-0.24	1.2	1.5	2.9
4	-0.12	2.7	0.80	-1.5	0.22	-0.8	-2.3	-3.1
5	0.65	-2.3	-0.02	3.0	-0.11	3.8	-1.9	1.9
6	-0.59	-5.2	0.72	-4.1	0.09	-0.9	0	-0.9
7	0.13	1.8	-0.51	0.3	0	-0.6	-5.1	-5.7
8	-0.17	4.3	-0.16	0.7	0.02	-1.6	6.8	5.0
9	-0.04	13.2	0.88	2.9	0.08	1.6	-3.7	-2.3
10	-0.07	-10.9	-0.65	0.1	-0.16	0	-5.4	-5.4
11	0.31	-12.3	-1.56	-4.9	0.03	-4.6	7.6	3.2
12	-0.21	11.0	1.38	2.3	0.10	3.0	1.5	4.6
13	0.20	-15.9	-1.65	-0.9	-0.33	-1.8	6.5	4.9
14	0.59	29.3	0.94	2.6	0.31	0.5	6.1	6.6
15	-0.56	6.8	1.45	-0.2	0.37	-0.6	-7.5	-8.0
16	-0.23	-20.4	-0.81	-1.6	-0.35	0.8	-4.6	-4.1

regression equation is as follows, the assumption being that the various ions act independently of each other:

$$X_1 = a' + f(X_2) + f(X_3) + f(X_4) + f(X_5) + f(X_6) + f(X_7)$$

In these analyses, the X_1 values are the weights of the various nitrogen and carbohydrate fractions found, as listed in tables 1, 2, 5, and 6. Of the in-

TABLE 4

Z' values, or residuals of the statistical analysis of the nitrogen fractions in corn leaf tissue

TREAT- MENT NUMBER	NH ₄ -N _i	NO ₃ -N	AMIDE N	AMINO N	BASIC N	SOLUBLE ORGANIC N	PROTEIN N	TOTAL ORGANIC N
1	-1.04	-14.1	0.85	-1.0	-0.03	-0.1	-16	-14
2	0.29	3.0	0	2.5	0	5.5	-7	0
3	0.58	7.6	-0.53	-1.8	-0.09	2.0	20	22
4	0.16	3.7	-0.32	0.1	0.12	-7.5	1	-8
5	-0.18	13.6	1.27	5.4	0.03	-3.6	9	8
6	-0.10	-9.3	-1.60	-2.4	-0.07	0.4	13	17
7	0.06	-3.1	0.63	-1.6	-0.03	5.0	-21	-16
8	0.23	-1.8	-0.34	-1.3	0.09	-2.6	-9	-9
9	-0.19	9.0	0.44	1.0	0.07	3.4	-4	0
10	-0.19	-7.7	-0.84	-2.9	0.07	-2.8	12	9
11	-0.60	-12.3	-0.51	-6.2	0.04	-5.1	-16	-20
12	1.00	11.4	0.96	8.4	-0.09	5.2	8	11
13	-1.21	-12.0	-1.27	-6.7	0.02	-2.6	11	13
14	1.31	31.1	1.58	6.3	0.05	2.4	-11	-11
15	0.22	-8.8	-0.20	2.4	0.10	6.6	-14	-11
16	-0.37	-10.4	-0.10	-2.0	-0.16	-6.4	18	9

TABLE 5

Carbohydrate fractions in corn stem tissue (X_i) and residuals from the statistical analyses (Z'_i), expressed in milligrams of glucose equivalent per 100 gm. fresh tissue

TREAT- MENT NUMBER	REDUCING SUGARS		SUCROSE		STARCH, DEXTRINS, ETC.		HEMICELLULOSE		TOTAL AVAILABLE CARBOHYDRATES	
	X ₁	Z' ₁	X ₁	Z' ₁	X ₁	Z' ₁	X ₁	Z' ₁	X ₁	Z' ₁
1	945	193	162	11	116	-2	848	107	2,071	310
2	729	-185	138	-48	144	7	854	-60	1,865	-286
3	670	242	229	31	119	17	681	46	1,699	337
4	703	-251	151	8	109	-24	854	-92	1,817	-361
5	580	-257	153	-52	235	-10	640	-67	1,608	-383
6	1,320	159	173	15	262	1	855	42	2,610	220
7	1,510	147	170	20	298	22	1,085	67	3,063	256
8	1,285	-38	210	17	270	-10	943	-43	2,708	-72
9	750	10	206	64	162	-14	890	-32	2,008	28
10	632	54	140	33	158	3	750	1	1,680	90
11	367	113	82	-72	121	-18	621	-22	1,191	0
12	593	-187	70	-29	199	29	1,005	51	1,867	-140
13	1,460	293	135	7	129	-28	967	-27	2,691	243
14	560	-405	74	-62	139	-3	639	-150	1,412	-619
15	544	-97	272	89	136	10	726	43	1,678	46
16	1,337	210	138	-33	177	16	1,098	136	2,750	329

dependent variables, X₂ represents the nitrate, X₃ the potassium, X₄ the phosphate, X₅ the calcium, X₆ the sulfate, and X₇ the magnesium concentrations in the substrate. The factor α' , again included in the values found for X'₂ in

the analyses, represents the statistically isolated effect of the nitrate level in the substrate upon the nitrogen or carbohydrate fraction under consideration found in the tissue.

Because of this inclusion of the a' factor in the X'_2 values, the net regression curves representing the effect of concentration of NO_3^- upon the amount of each fraction found, are determined to be all positive values. The X'_3 to X'_7 values, represented by separate curves, are all corrections to be applied to the nitrate curves. These corrections are additive, depending upon the solution concentrations actually employed, and their accuracy is a function of the residuals, or Z'_7 values, listed in tables 3-6. The various X' values may be read directly from the regression curves.

TABLE 6

Carbohydrate fractions in corn leaf tissue (X_1) and residuals from the statistical analyses (Z'_7), expressed in milligrams of glucose equivalent per 100 gm. fresh tissue

TREAT- MENT NUMBER	REDUCING SUGAR		SUCROSE		STARCH, DEXTRINS, ETC.		HEMICELLULOSE		TOTAL AVAILABLE CARBOHYDRATES	
	X_1	Z'_7	X_1	Z'_7	X_1	Z'_7	X_1	Z'_7	X_1	Z'_7
1	389	92	143	23	163	-11	3,140	101	3,835	201
2	239	-39	118	-1	250	-27	3,490	-182	4,097	-246
3	247	76	166	27	177	42	2,920	106	3,510	251
4	251	-128	137	-50	268	-3	3,650	-24	4,306	-206
5	359	-171	176	2	300	25	3,100	10	3,935	-139
6	778	122	179	24	352	18	3,180	-135	4,489	40
7	880	142	152	-70	379	-32	4,000	50	5,411	84
8	547	-90	196	42	406	-11	4,020	72	5,169	11
9	267	-5	141	-52	475	10	3,640	-62	4,523	-104
10	204	-87	187	-7	405	43	3,370	301	4,166	248
11	339	174	181	-32	263	-60	2,560	-284	3,343	-200
12	288	-85	352	91	466	7	3,750	46	4,856	60
13	800	77	281	29	424	30	3,510	-72	5,015	63
14	513	-128	143	-42	249	-48	2,680	-267	3,585	-489
15	437	-78	209	5	250	-8	2,890	168	3,786	87
16	753	131	191	7	427	27	3,750	170	5,121	338

Partial correlation coefficients have not been calculated. The absolute reliability of the net regression curves is not known, therefore; but since interpretation of the curves will be confined to obvious trends, the significance of the curves should not be impaired.

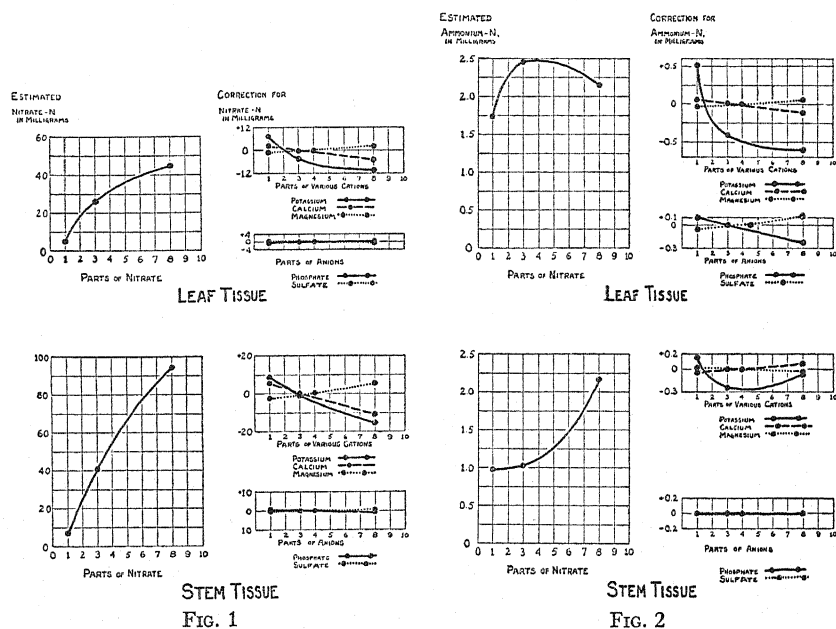
Nitrogen fractions

Figure 1 represents the effect of the concentrations of the nutrient ions in the substrate upon the nitrate nitrogen content of the tissue. Nitrate nitrogen in the tissue varies directly with the NO_3^- concentration and inversely with the Ca^{++} and with the K^+ concentration of the substrate. It is suggested that these latter inverse variations, which will be further discussed later in the

paper, are associated with an increased growth rate resulting from a favorable ionic balance in the substrate.

The net regression curves representing the accumulation of ammonium nitrogen in the tissue, shown in figure 2, are based upon statistical analysis which left disproportionately large Z' values. Nevertheless, the ammonium content of the tissue varies directly with the nitrate concentration of the substrate and inversely with the K^+ concentration.

There is a pronounced positive correlation between the NO_3^- concentration of the substrate, as well as a negative correlation between the K^+ concentration



FIGS. 1 AND 2. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF NITRATE NITROGEN (FIG. 1) AND AMMONIUM NITROGEN (FIG. 2) IN CORN TISSUE

The nitrogen found is expressed in milligrams per 100 gm. fresh tissue

of the substrate, and the α -amino nitrogen content of the tissues. The amino nitrogen fraction comprises a considerable part of the total soluble nitrogen, as seen in tables 1 and 2. Variations in the concentration of K^+ are every bit as effective as variations in the NO_3^- concentration upon the α -amino nitrogen content of the tissue, although the effects are opposite in sign.

Correlations similar to those in figures 1, 2, and 3 are shown in figure 4 with respect to the relation between substrate ion concentration and the amide nitrogen content of the tissues.

The effects of ionic substrate concentrations upon the basic nitrogen content of the tissues are graphically represented in figure 5. The chemical com-

position of this nitrogen fraction is not known, and its metabolic significance is not well understood. It is not generally considered to be a fraction essential in the direct path of protein synthesis. There is a direct correlation between high NO_3^- concentration in the substrate and high basic nitrogen content of the tissue, but variations in the substrate concentrations of the other nutrient ions do not affect the tissue content of this nitrogen fraction significantly.

The protein nitrogen fraction (fig. 6) represents a large proportion of the nitrogen of the plant, and the small Z'_7 values of tables 3 and 4 indicate that the statistical analysis is highly accurate. Here, again, there are significant correlations. The NO_3^- concentration of the substrate has no apparent effect upon

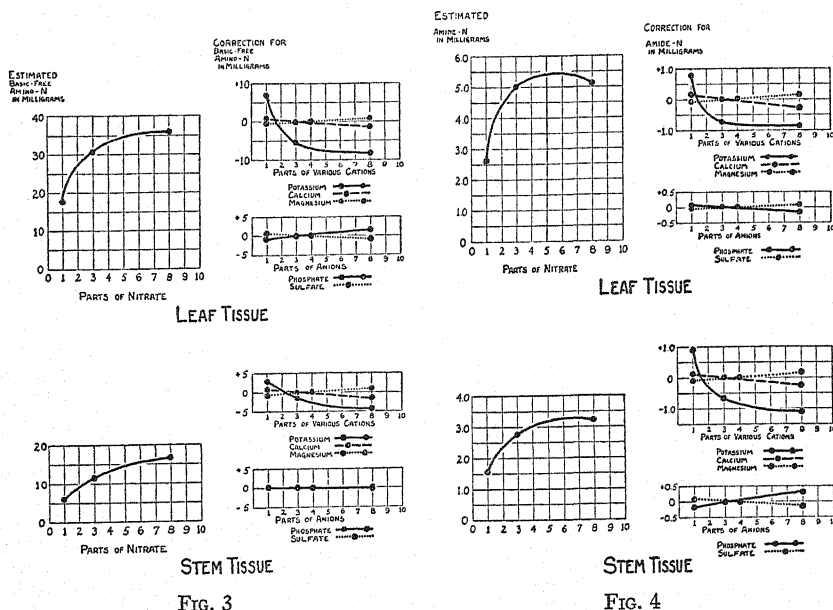


FIG. 3

FIG. 4

FIGS. 3 AND 4. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF α -AMINO NITROGEN (FIG. 3) AND AMIDE NITROGEN (FIG. 4) IN CORN TISSUE

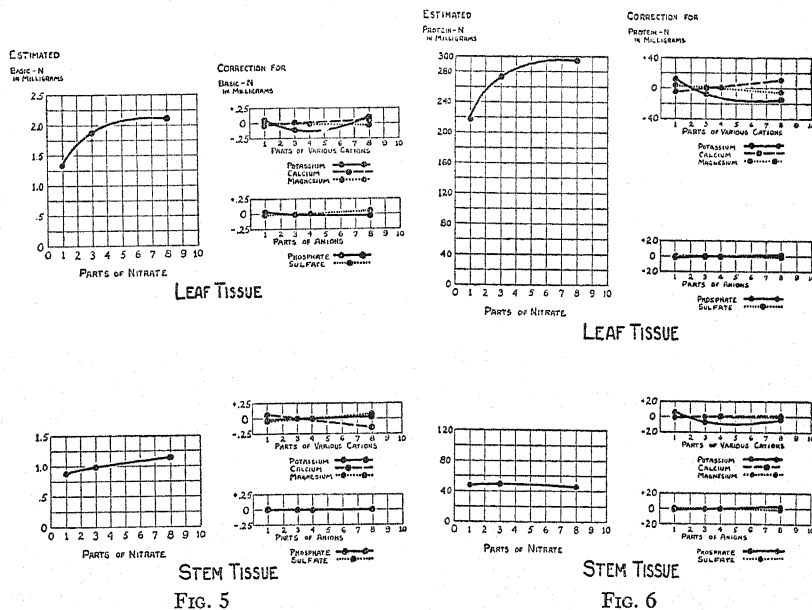
The nitrogen found is expressed in milligrams per 100 gm. fresh tissue

the amount of protein in the stem tissue but is positively correlated with the protein content of the leaf tissue. Increasing K^+ concentrations in the substrate are again inversely related to the protein content. Increasing Ca^{++} concentrations are associated with slightly increasing protein content of the leaf tissue, but there is no such correlation in the case of the stem tissue. Inasmuch as the variations in protein content due to the level of concentration of K^+ or Ca^{++} in the substrate are very small with respect to the total protein content, it is probable that they represent fluctuations in the storage proteins only, or that they may be due to variations in the water content of the tissues.

Figures 7 and 8 show correlations with nutrient ion concentrations of soluble

organic and total organic nitrogen fractions, each of which includes heterogeneous nitrogenous fractions, and are here presented in order to emphasize relationships previously discussed. They serve also to emphasize the fact that the effects of the cations are more pronounced upon the soluble than upon the insoluble nitrogenous fractions.

It is evident from the data that variations in the nutrient concentrations of the $\text{PO}_4^{=}$, $\text{SO}_4^{=}$, and Mg^{++} ions within the limits employed do not significantly affect the tissue content of the nitrogenous fractions determined. The lowest concentrations of each of these ions in the nutrient solutions exceeded the concentrations below which external symptoms of the deficiency of the respective ions usually occur.



FIGS. 5 AND 6. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF BASIC NITROGEN (FIG. 5) AND PROTEIN NITROGEN (FIG. 6) IN CORN TISSUE

The nitrogen found is expressed in milligrams per 100 gm. fresh tissue

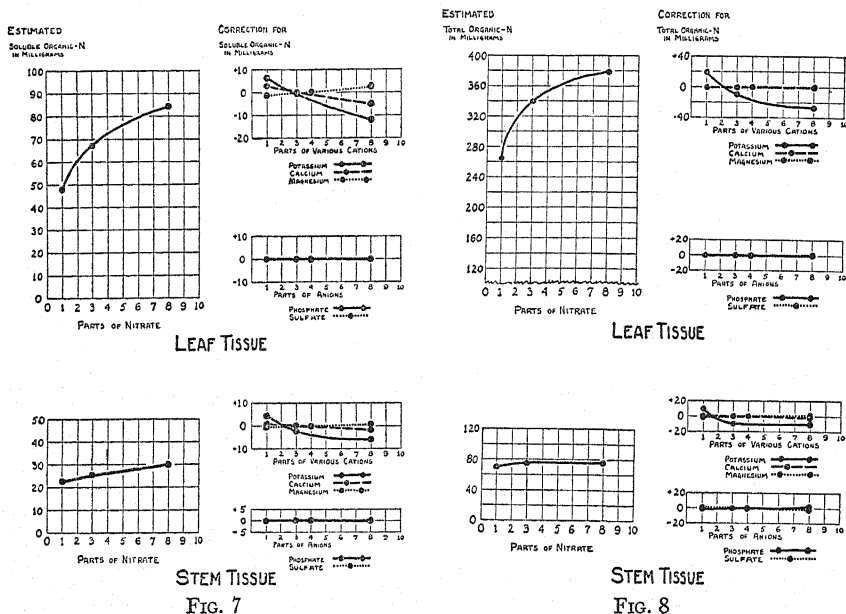
Carbohydrate fractions

It is well known that the plant requires large amounts of energy in the synthesis of proteins and especially in the reduction of nitrates and that the source of this energy is the oxidation or dehydrogenation of available carbohydrates. No study of nitrogen metabolism is really complete without a consideration of this energy source, since in healthy plants the rate of energy supply is probably the most important single factor concerned with the rate of protein synthesis and growth.

In corn plants, hexose sugars are generally considered as the carbohydrates utilized as the energy source. Sucrose has been considered particularly important in translocation and storage, and the more highly condensed non-soluble carbohydrates function principally as storage products.

Although convenient methods used for separating the carbohydrate fractions are not specific, the separation methods used in this work were considered adequate for comparisons.

Figure 9 represents the net regression curves determined from the reducing sugar analyses. Each curve represents the isolated effect of the concentration of each ion in the substrate upon the reducing sugar concentration found in the



FIGS. 7 AND 8. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF SOLUBLE ORGANIC NITROGEN (FIG. 7) AND TOTAL ORGANIC NITROGEN (FIG. 8) IN CORN TISSUE

The nitrogen found is expressed in milligrams per 100 gm. fresh tissue

tissue. Many studies of nitrogen-carbohydrate metabolism have shown that with a high nitrogen supply the total carbohydrate content of plant tissue is relatively low. The reducing sugar content of the tissues of such plants has often been found to be relatively high, but on the other hand sometimes has been found to be relatively low. Factors usually undetermined are evidently responsible for these differences. Figure 9 shows that in the present experiment the reducing sugar content of both the leaf and stem tissues decreased with increasing NO_3^- concentrations in the substrate. An important factor in this connection may be the relative K^+ concentration of the substrate. Figure 9 shows that increases in the K^+ concentration in the substrate were

associated with decreases in the reducing sugar content of the tissue, and that these decreases were comparable in magnitude with those caused by increases in the NO_3^- concentration. In many studies of nitrogen nutrition, the physiological significance of the ion accompanying the NH_4^+ or NO_3^- is ignored in formulating the composition of solution used in varying the nitrogen levels. Consequently, observed variations in reducing sugar content of the tissues may be ascribed entirely to variations in nitrogen concentrations, whereas in reality these variations may be considerably influenced by variations in relative concentrations of the accompanying ion. The importance of cation balance in connection with the growth and chemical composition of these plants has been discussed in two previous papers (2, 3).

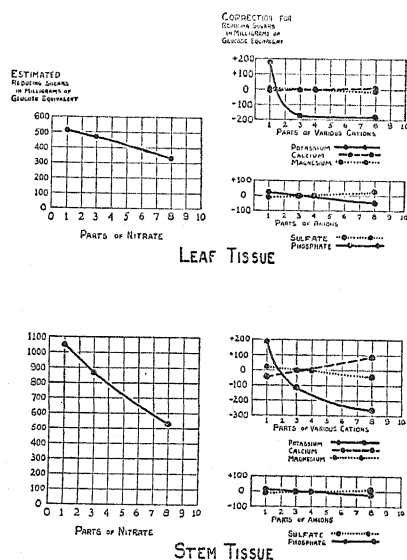


FIG. 9. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF REDUCING SUGARS IN CORN TISSUE

The reducing sugars found are expressed in milligrams of glucose equivalent per 100 gm. fresh tissue

The importance of cation balance with respect to the relation between nitrogen content of the substrate and reducing sugar content of the tissue is evident if potassium is a regulating factor, direct or indirect, in energy release and glucose oxidation.

There is some evidence in the field of animal biochemistry that this is the case, and that potassium functions either as a co-catalyst with enzymes or as a catalyst in the formation of enzymes, which in turn bring about energy release in the breakdown of glucose. It has long been observed that potassium is closely correlated with irritability in animal tissue, and that the concentration

of potassium in nerve and brain tissues is very high. Ashford and Dixon (1), working with rabbit brain slices, found that the presence of 0.1 *M* KCl greatly increases the production of lactic acid from glucose, in the presence of oxygen.

REDUCING SUGARS

MILLIGRAMS GLUCOSE EQUIVALENT
PER 100 GRAMS FRESH TISSUE

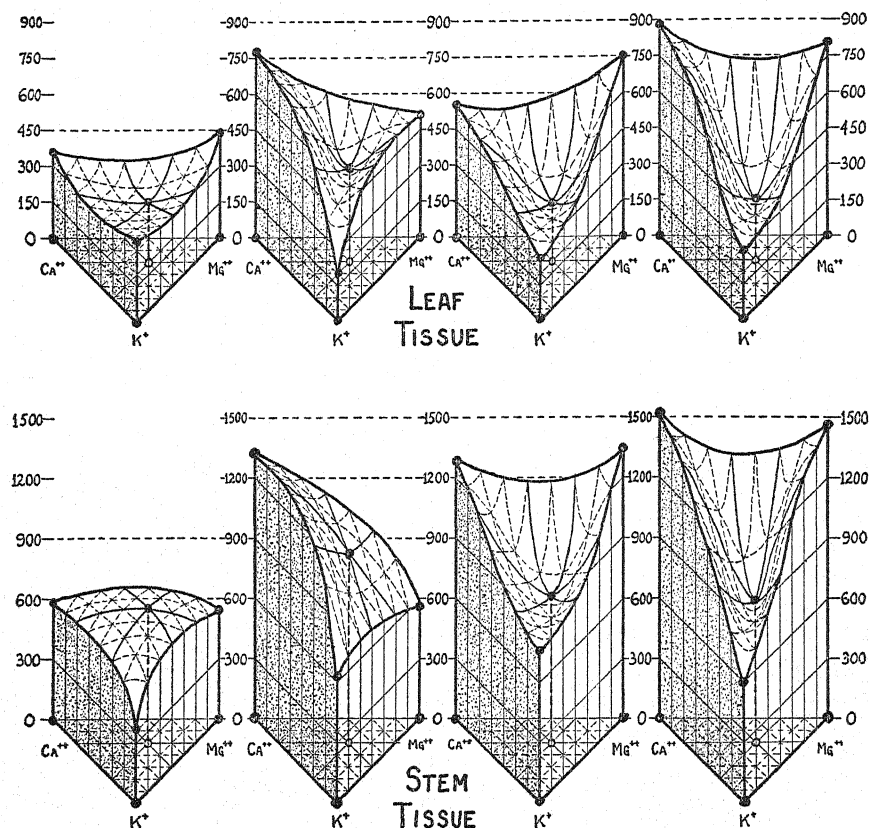


FIG. 10. INTERRELATIONSHIP OF CONCENTRATIONS OF THE VARIOUS CATIONS, AS THEY INFLUENCE REDUCING SUGARS FOUND

The prisms, left to right, represent high nitrate (nitrate 8 parts, sulfate 1 part, and phosphate 1 part), medium nitrate (nitrate 3 parts, sulfate 4 parts, and phosphate 3 parts), low nitrate (high phosphate), and low nitrate (high sulfate). The cation distribution indicated at the corners of each prism represents high concentration where there is a substrate concentration of 8 parts of the cation indicated and 1 part of each of the two other cations.

Under low potassium conditions in plant tissue, increase in glucose concentration despite added nitrogen might be accounted for if this specific energy release role in plant metabolism be assigned to potassium. Such a role for

potassium in plant metabolism likewise might account for the relatively high potassium requirement of meristematic tissues.

The relations between the nutrient concentrations of NO_3^- and K^+ and the reducing sugar content of the tissues are shown diagrammatically in figure 10. The reducing sugar content of the tissue is plotted at four points, in each prism diagram, against the relative concentration of each of the three cations in the substrate. The form of the curves which limit the upper surface was determined by estimation, using the locations of the central point on the upper surface and the upper points on the prism diagrams as reference points. It was felt that an estimate of the probable location of the internal points of these

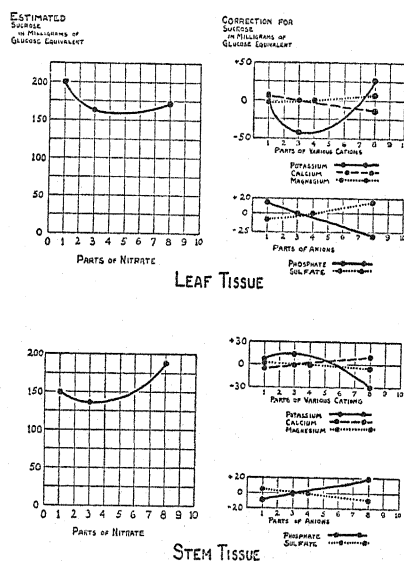


FIG. 11. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF SUCROSE IN CORN TISSUE

The sucrose found is expressed in milligrams of glucose equivalent per 100 gm. fresh tissue

curves would be much nearer to their actual location than would straight line connections. The distance of this upper surface from the base at any point is a measure of the tissue content of reducing sugars found.

A consideration of figure 10 shows that regardless of the variations in reducing sugars found under other nutritional conditions, high K^+ concentration is definitely correlated with low reducing sugars, at any level of NO_3^- in the substrate.

Returning to the consideration of the nitrogen fraction data, and bearing in mind that the tissue contents of all those fractions directly concerned with the synthesis of proteins decrease with increasing potassium concentration in the

substrate, we find here a substantiation of the close relationship between the K^+ concentration and energy release in the tissue. It is more logical to assume that the influence of potassium in all these nitrogen assimilation processes is

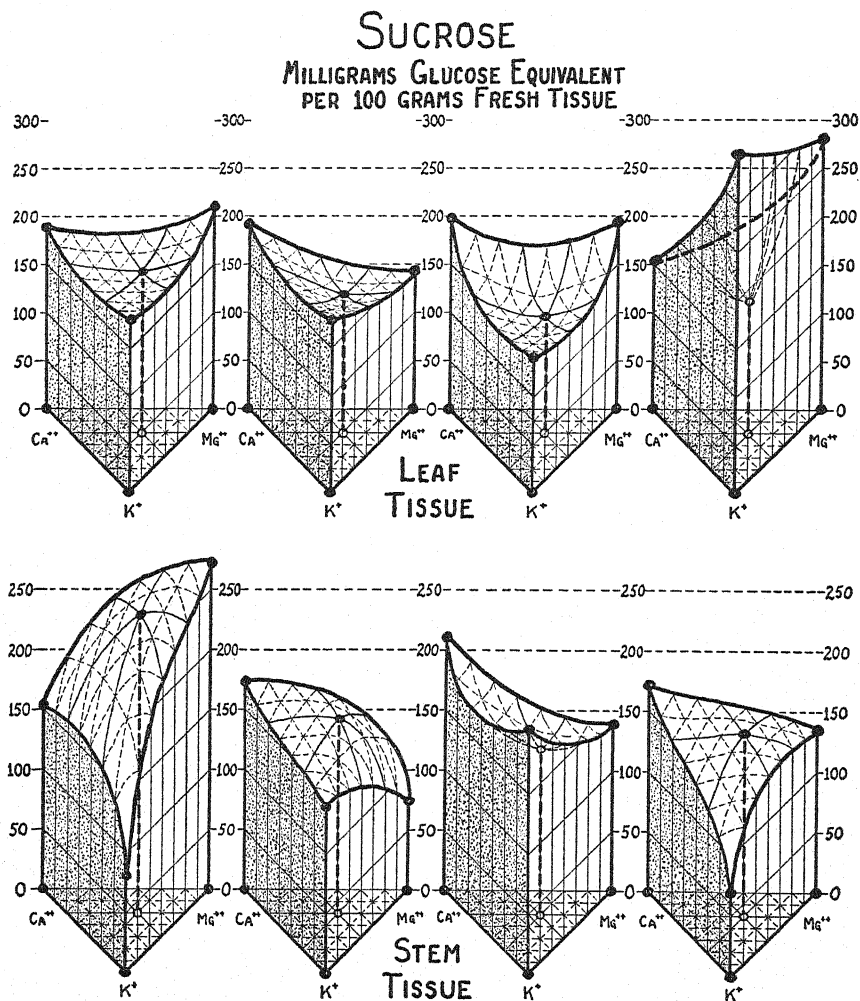


FIG. 12. INTERRELATIONSHIP OF CONCENTRATIONS OF THE VARIOUS CATIONS, AS THEY INFLUENCE THE SUCROSE FOUND

The prisms, left to right, represent high nitrate, medium nitrate, low nitrate (high phosphate), and low nitrate (high sulfate). The cation distribution indicated at the corners of each prism represents high concentration.

due indirectly to the release of energy in the tissues, which in turn is associated with the assimilation of nitrogen to proteins, than it is to consider that the potassium ion catalyzes directly every step in these synthetic processes. This

conception of the major function of potassium in metabolic processes is suggested by these data.

A consideration of figures 11 and 12, which show the relations between the ionic substrate concentrations and the sucrose content of the tissue, leads to no definite conclusions. Sucrose represents a translocatory and a temporary storage form of carbohydrates in corn tissue, and its relative concentration in the tissues is not readily correlated with the variations of nutrient ions. Das (5) and others have found a negative correlation between the sucrose concentration and the hydration of the tissue, and this correlation may be important in connection with the interpretation of these results.

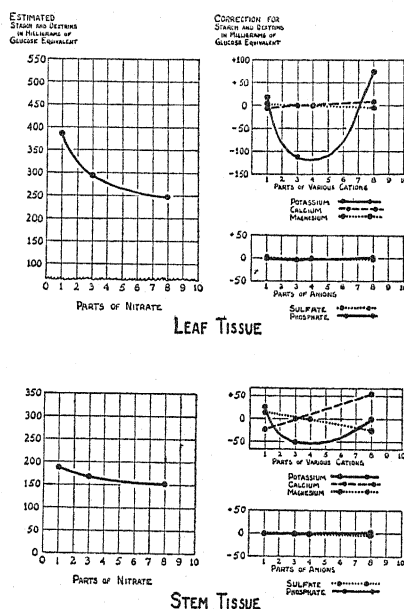


FIG. 13. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF STARCH AND DEXTRINS IN CORN TISSUE

The starch and dextrins found are expressed in milligrams of glucose equivalent per 100 gm. fresh tissue

Figures 13 to 16 showing the effect of the nutrient ions upon starch and dextrin and hemicellulose accumulation are of interest for three reasons: first, there is a tremendous dip in the regression curve for the effect of potassium on the starch and dextrin content of the tissues; second, aside from this dip, high and low proportions of K^+ show no significant effect on the concentration of the reserve carbohydrates; third, the figures indicate that calcium may function in some manner in the accumulation of storage carbohydrates.

In regard to the first point of interest, according to the method by which the nutrient solutions were prepared, when K^+ was present in the substrate at

three relative parts, the concentrations of Ca^{++} and Mg^{++} were fixed at three and four relative parts respectively (3). Consequently, since the statistical allowance to be made for potassium was removed first (the effect of K^+ con-

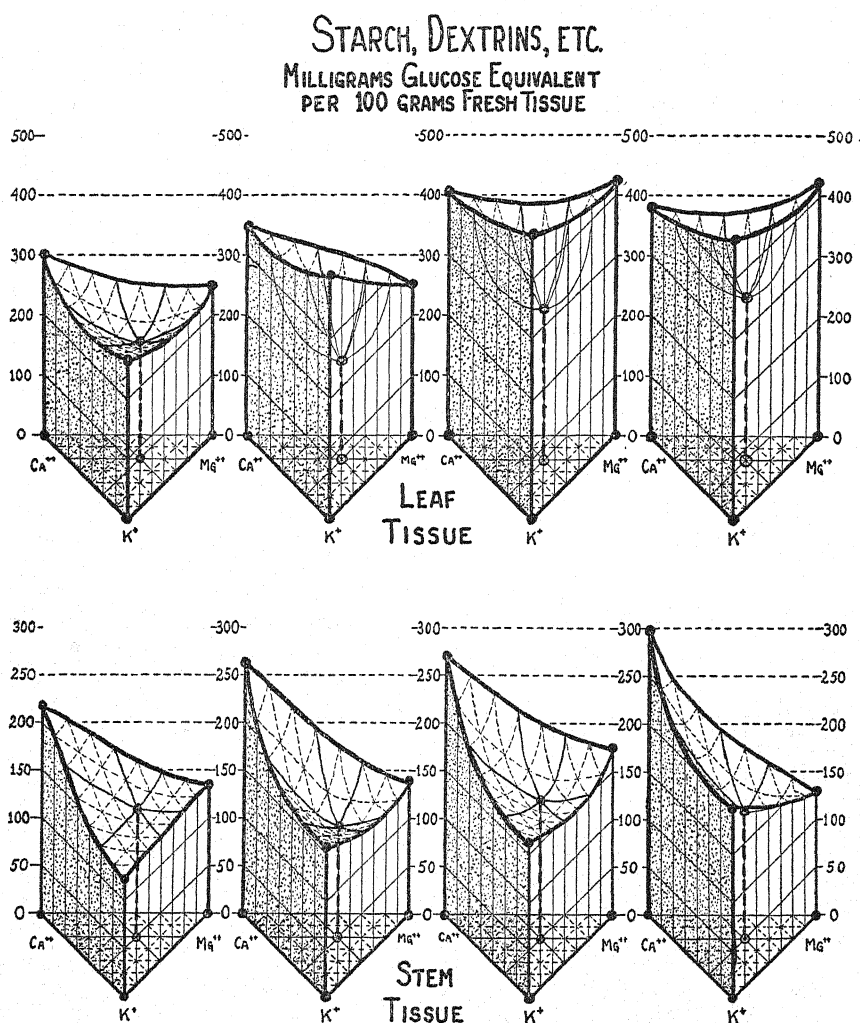


FIG. 14. INTERRELATIONSHIP OF CONCENTRATIONS OF THE VARIOUS CATIONS, AS THEY INFLUENCE THE STARCH AND DEXTRINS FOUND

The prisms, left to right, represent high nitrate, medium nitrate, low nitrate (high phosphate), and low nitrate (high sulfate). The cation distribution indicated at the corners of each prism represents high concentration.

centration was represented by the X_2 value), the allowance for Ca^{++} concentration at three relative parts and for Mg^{++} concentration at four relative parts was removed with it. The dip in the curve showing the effect of potas-

sium (fig. 13) may be associated with the cation balance of the nutrient medium, but its metabolic significance is not clear from this method of analysis. The actual picture with respect to the effect of nutrient ions upon starch and dextrin content of the tissues is clearer in figure 14.

The second interesting point concerning the regression curves of figures 13 to 16 is particularly obvious with regard to the effect of K^+ concentration upon the hemicellulose content of the tissue (figs. 15, 16). An explanation of this lack of significant effect is suggested by a consideration of the curves representing the effect of the nitrate concentration upon the tissue content of the carbohydrate fractions. It was suggested previously that the relative potassium

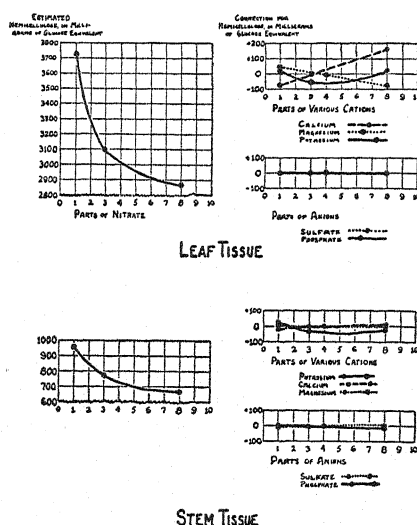


FIG. 15. NET REGRESSION CURVES SHOWING THE EFFECTS OF CONCENTRATION OF THE VARIOUS IONS IN THE SUBSTRATE UPON ACCUMULATION OF HEMICELLULOSES IN CORN TISSUE

The hemicelluloses found are expressed in milligrams of glucose equivalent per 100 gm. fresh tissue

concentration conditions the rate of energy release through hexose sugar oxidation, which in turn conditions the rate of nitrogen assimilation. Since the rate of nitrogen assimilation cannot proceed more rapidly than the rate at which nitrogen is supplied for assimilation, this latter factor conditions the whole process, and in the final analysis, the reserve of carbohydrates therefore is dependent largely upon the concentration of the available nitrogen. It is believed that this is the principal reason why the role of potassium has so long been obscure. The function of potassium must be studied through its effect upon the immediate energy source, hexose sugars. It is also essential that the effect of different concentrations of potassium should be studied simultaneously at several different levels of nitrogen supply, in order to obtain a

true picture of the effect of K^+ concentration upon carbohydrate-nitrogen relationships in the plant.

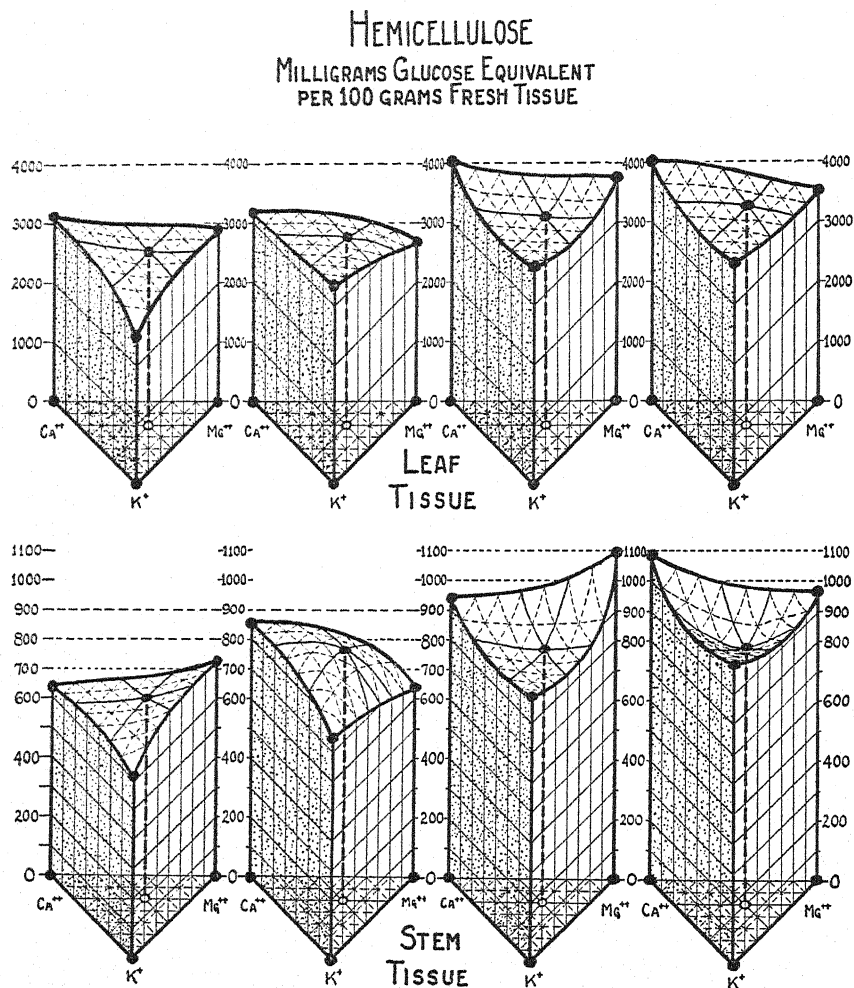


FIG. 16. INTERRELATIONSHIP OF CONCENTRATIONS OF THE VARIOUS CATIONS, AS THEY INFLUENCE THE HEMICELLULOSES FOUND

The prisms, left to right, represent high nitrate, medium nitrate, low nitrate (high phosphate), and low nitrate (high sulfate). The cation distribution indicated at the corners of each prism represents high concentration.

In view of the high negative correlation between K^+ concentration in the substrate and reducing sugar content of the tissue, it is not clear at present why a similar correlation was not found to exist between the potassium concentration and carbohydrate reserves. If, however, these reserve carbohydrates

were formed more rapidly in the high potassium tissues than in the low potassium tissues, this might account for the lack of close correlation between the K^+ concentration and reserve carbohydrate content. In this connection, any role of potassium which directly or indirectly increases the efficiency of carbon assimilation would therefore account for the rather high accumulation of storage carbohydrates in the high potassium tissues without necessarily affecting the content of reducing sugars. Many studies have already indicated the importance of the role of potassium in this regard.

Finally, though figures 13-16 indicate that calcium may function in the accumulation of storage carbohydrates, the manner in which it functions is certainly not evident from these data. But since it has been established by a statistical correlation, not included in this paper, that high calcium tissues are relatively higher in per cent dry weight than are high potassium or high magnesium tissues, it is possible that this may have the effect of shifting the carbohydrate equilibria from simple sugars toward the storage carbohydrate condition.

SUMMARY

Carbohydrate and nitrogen fraction relationships were interpreted statistically in an attempt to establish correlations between the concentrations of the various nutrient ions in the substrate and the status of nitrogen metabolism with tissues of corn, grown in sand culture using a variable ion proportion series. All calculations were based on fresh weight data.

Within the limits used, $PO_4^{=}$ and $SO_4^{=}$ concentrations were found to have no demonstrable correlation with nitrogen metabolism.

Increasingly high NO_3^- concentrations in the substrate were found to increase the content of nitrate, ammonium, basic-free α -amino, amide, basic, and protein nitrogen in the tissues.

Increasingly high concentrations of NO_3^- in the substrate were found to decrease the tissue content of reducing sugars, starch and dextrans, and hemicelluloses.

Increasingly high concentrations of Ca^{++} in the substrate were found to decrease slightly the soluble nitrogen fractions, to increase slightly the protein nitrogen content, and to increase the complex reserve carbohydrate content of the tissues. No significant correlations were found between Ca^{++} in the substrate concentration and the reducing sugar and sucrose content of the tissues.

Increasingly high Mg^{++} concentrations within the limits used were found to have no appreciable effect upon carbohydrate-nitrogen relationships.

Increasingly high K^+ concentrations in the substrate were found to have little effect upon protein or basic nitrogen content of the tissues but were found to decrease the tissue content of all other soluble nitrogen fractions. Increasingly high K^+ concentrations in the substrate were found to decrease, also, the content of reducing sugars in the tissue but to have no appreciable effect upon sucrose, starch and dextrin, or hemicellulose content of the tissue.

As a result of these experiments it is suggested that potassium may be essential to the processes by which energy utilized in nitrogen metabolism is released from the simple sugars.

REFERENCES

- (1) ASHFORD, C. A., AND DIXON, K. C. 1935 The effect of potassium on the glucolysis of brain tissue with reference to the Pasteur effect. *Biochem. Jour.* 29: 157-168.
- (2) BECKENBACH, J. R., ROBBINS, W. R., AND SHIVE, J. W. 1938 Nutrition studies with corn: II. A statistical interpretation of the relation between the ionic concentration of the culture solutions and the element content of the tissues. *Soil Sci.* 45: 403-426.
- (3) BECKENBACH, J. R., WADLEIGH, C. H., AND SHIVE, J. W. 1936 Nutrition studies with corn: I. A statistical interpretation of the nutrient ion effect upon growth in artificial culture. *Soil Sci.* 41: 469-489.
- (4) CHIBNALL, A. C. 1922 Investigations on the nitrogenous metabolism of the higher plants: II. The distribution of nitrogen in the leaves of the runner bean. *Biochem. Jour.* 16: 344-362.
- (5) DAS, U. K. 1936 Nitrogen nutrition of sugar cane. *Plant Physiol.* 11: 251-317.
- (6) EZEKIEL, M. 1930 Methods of Correlation Analysis. John Wiley & Sons, New York.
- (7) MORROW, C. A. 1927 Biochemical Laboratory Methods for Students of the Biological Sciences. John Wiley & Sons, New York.
- (8) NIGHTINGALE, G. T., ROBBINS, W. R., AND SCHERMERHORN, L. G. 1927 Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. *N. J. Agr. Exp. Sta. Bul.* 448.
- (9) OSBORNE, T. B., AND HARRIS, I. F. 1903 Nitrogen in protein bodies. *Jour. Amer. Chem. Soc.* 25: 323-353.
- (10) RANKER, E. R. 1925 Determination of total nitrogen in plants and plant solutions: A comparison of methods with modification. *Ann. Missouri Bot. Gard.* 12: 367-380.
- (11) SESSIONS, A. C., AND SHIVE, J. W. 1928 A method for the determination of inorganic nitrogen in plant extracts. *Plant Physiol.* 3: 499-511.
- (12) TOMPSETT, S. L. 1930 The determination of blood-sugar: I. Critical analysis of the reduction of alkaline copper reagents by glucose and other substances. *Biochem. Jour.* 24: 1148-1163.

PHYSICAL CHARACTERISTICS OF SOILS: VI. INFLUENCE OF CLAY, EXCHANGEABLE BASES, AND HYGROSCOPIC MOISTURE ON SOIL COHESION

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A new method of determining soil cohesion, as outlined in a previous paper (3), consists in measuring the force required to break a semicircular pellet 1 inch in diameter, the load being applied on the center of the curved surface. A minor modification in the technic has been to use a steel ball 1 inch in diameter instead of a flat surface for applying the force. This ensures a more uniform distribution of the load through a point contact.

INFLUENCE OF CLAY

Clay is the most important constituent of soils, for it acts as the binder and is almost entirely responsible for the cohesion in dry soils. The magnitude of the force of cohesion must depend on the points of contact between the particles. Obviously, the smaller the particles, the more numerous are the points of contact in a given weight of the most closely packed soil. This pertains, of course, only to spherical particles, but the argument will not be materially affected in the case of sand particles of irregular shape for purposes of comparison. Clay acts like glue in binding the particles together, and it would be interesting to know how this cementing action is influenced by the size of the particles.

The effect of increasing amounts of clay on the cohesion in silt (average diameter 0.0764 mm.) and sand (average diameter 0.2587 mm.) was studied. The results are plotted in figure 1. It will be seen that the cementing action for a given percentage of clay is greater in silt than in sand, though the maximum value is the same in both cases. Another point worth noting is that beyond a certain limit, further additions of clay do not increase cohesion. This condition is evidently reached when all the spaces between the larger particles have been filled with clay, so that any further increase merely pushes these particles apart, in which case we are, in fact, dealing with cohesion between clay particles which have completely enveloped the larger particles. It is obvious from these results that a much larger amount of clay binder will be required for sand than for silt to attain maximum strength. In order, therefore, to save as much of the valuable binder material as possible, the voids between the larger particles must be filled with smaller particles of a gradually diminishing size.

In order to obtain fuller information on the exact relation between the size

of particles and changes in cohesion due to the addition of clay, sand and silt particles of various sizes were separated by means of Puri siltometer (2) and beaker sedimentation respectively. To the various fractions increasing

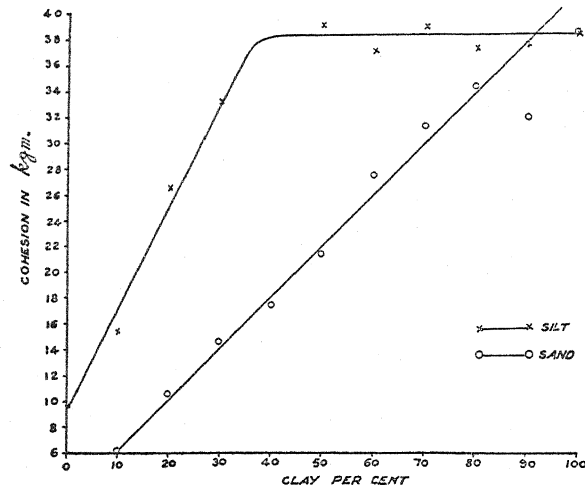


FIG. 1. RELATION BETWEEN COHESION AND THE CLAY CONTENT OF SILT AND SAND

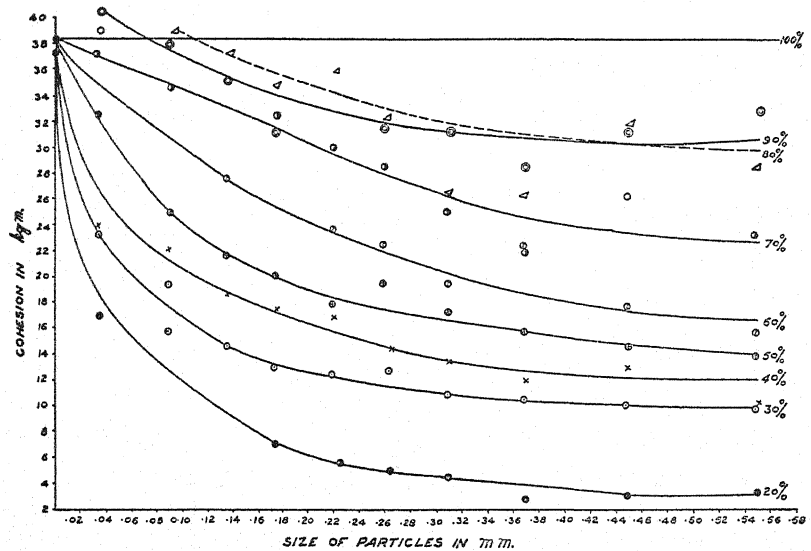


FIG. 2. EFFECT ON COHESION OF CLAY SOIL OF PERCENTAGE OF CLAY AND SIZE OF SAND AND SILT PARTICLES

amounts of clay were added, and cohesion was measured. The clay was from an alluvial deposit containing 89 per cent particles below 0.002 mm. diameter, and only 5 per cent above 0.02 mm. diameter. It was not considered

necessary to separate the conventional clay fraction, and the whole of it was taken as clay for purposes of comparison. The results are plotted in figure 2. It will be seen that cohesion for the same percentage of clay rapidly falls as the particle size increases to a limit beyond which the effect of size of particles becomes negligible. Another interesting point noticed in these results is that the maximum cohesion of 100 per cent clay is of the order of 38 kgm. This value is by no means the maximum for clays of different types. In fact, many soils containing much less clay show higher values. It is for this reason that the relation between clay and cohesion in natural soils is only qualitative.

EFFECT OF HYGROSCOPIC MOISTURE AND EXCHANGEABLE BASES

Effect of moisture on soil cohesion has been studied chiefly in the wetter regions, where it is entirely accounted for by the surface tension of the liquid films of decreasing thickness which draws the particles closer and closer. In the regions of hygroscopic moisture, drying or wetting leads to very little change in volume of the soil as a whole, and consequently the limit of compactness has been reached and the particles can draw no closer together on further drying. This region, therefore, seems to have presented no point of interest, and information regarding it is confined to a few isolated observations. The enormous change in cohesion due to the drying of the hygroscopic moisture leads one to the conclusion that beyond a certain degree of wetness the cohesive forces in soils are partly molecular and therefore might be associated with the nature of the exchangeable ion in the clay complex.

The influence of exchangeable bases and hygroscopic moisture was studied on single-base soils by first removing all the exchangeable bases by 0.05 *N* HCl treatment and then adding hydroxides of various metals. The soils were oven dried and then gradually allowed to take up moisture from atmospheres of different humidities in vacuum desiccators for 72 hours. In the soils to which NaOH and Ca(OH)₂ had been added, the relation between moisture and cohesion was also studied by gradually drying the soils. The results are plotted in figure 3, from which the following conclusions may be drawn:

The effect of exchangeable bases on soil cohesion is maximum when the soil is dry. The absorption of moisture leads to a narrowing of the differences due to ions, which become negligible when the soil is in equilibrium with 90 per cent relative humidity.

The order of cohesion for the dry soil follows the generally accepted order of dissociation for these ions.

The relation between moisture content and cohesion is substantially the same, whether the soil is gradually dried or rewetted.

The greater cohesion in Na- and Li-soils, in comparison with other single-base soils, may be due to a stronger bond between the dissociated ions, or it may be caused by the enhanced dispersion of the clay particles. For instance, it can be shown that in Na- and Li-soils, the dispersion of clay is much greater than in the case of other ions. The high dispersion would result in a larger number of points of contact, and the cohesive forces would be greater. The

latter view is probably the correct one, for if the soil is first completely dispersed and then converted into a H-soil by acid treatment and different ions are introduced as hydroxides without allowing the soil to dry, the cohesion is enormously increased over that of undispersed soil. This will be seen from table 1 in which the effect of exchangeable ions on the dispersed and undispersed soils is compared. The cohesion was measured on the dry soils. The effect of cations even in the dispersed soils persists. This would seem to indicate that apart from the state of dispersion the cations may have a specific

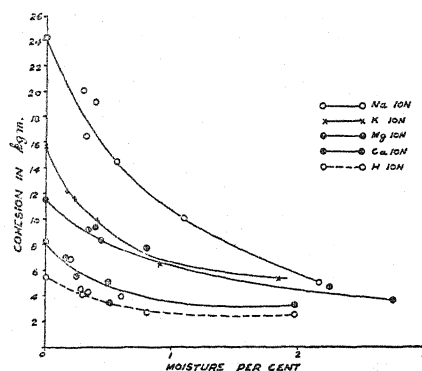


FIG. 3. EFFECT OF MOISTURE ON COHESION IN SINGLE-BASE SOILS

TABLE 1

Cohesion of soil as affected by various ions, by dispersion, and by remaking of broken pellets

IONS	UNDISPERSED SOIL		DISPERSED SOIL	
	Original pellet	Remade pellet	Original pellet	Remade pellet
	kgm.	kgm.	kgm.	kgm.
Li.....	32.3	40.1	44.5	54.6
Na.....	23.2	23.2	40.9	46.3
K.....	16.7	19.1	33.8	29.3
Mg.....	16.0	15.2	33.1	32.8
Ca.....	14.1	11.4	27.8	31.2
Sr.....	10.9	12.9	30.0	29.1
Ba.....	9.1	12.8	25.9	26.6
H.....	9.0	23.2	21.1

effect. It was noticed, however, in another study of the ultramechanical analysis of soils (5) that although the amount of conventional clay in a dispersed soil was the same whatever the nature of the cation introduced, this was not true of particles finer than clay, which showed a higher percentage in the case of Li and Na ions. These finer particles would easily account for the greater cohesion in Na- and Li-soils.

The effect of pH value on the cohesion of single-base soils was next studied by adding increasing amounts of the various hydroxides to a H-soil. The

results are shown in figure 4. The high cohesion of Na- and Li-soils is again brought out. It is seen that, in the case of Na and K, the cohesion reaches a maximum value, beyond which further additions of alkali result in a lowering of the cohesion. Li ions, on the other hand, show no such falling off in the cohesion. The difference is most probably due to the flocculating effect of NaOH and KOH, an effect which is absent in the case of LiOH. It might be mentioned that flocculating at these concentrations of NaOH has not been observed in suspensions, but at low moisture contents at which the cohesive forces

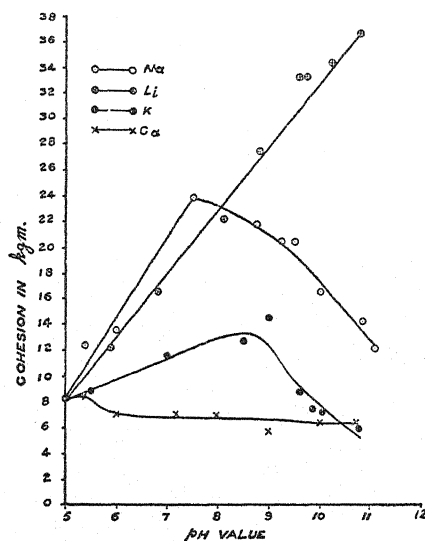


FIG. 4

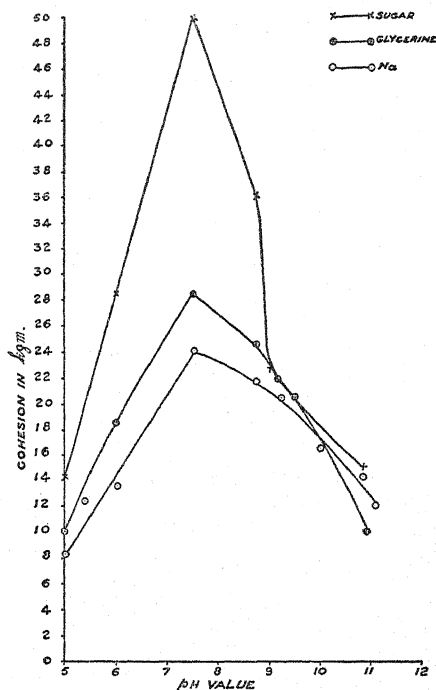


FIG. 5

FIG. 4. EFFECT OF pH VALUE ON COHESION OF SOIL WITH VARIOUS HYDROXIDES

FIG. 5. EFFECT OF pH VALUE ON COHESION OF SOIL IN THE PRESENCE OF SUGAR AND GLYCERINE

come into play, the concentration of the Na ions might easily go high enough to cause flocculation.

The effect of flocculation with CaCl_2 on cohesion of a sodium soil was also studied. The soil was first completely dispersed by shaking with Na_2CO_3 -NaOH, and then increasing amounts of CaCl_2 were added to the suspension until flocculation was observed. It must be understood that the flocculation value of the suspension may not correspond with the flocculation of the soil at low moisture content. The decrease in cohesion therefore could be expected to have occurred before flocculation was observed, when 8 m.e. CaCl_2

was added. The results given in table 2 show that the presence of CaCl_2 causes a substantial reduction in the cohesion of the dry soil.

The effect of calcium silicate on the cohesion of the soil must be distinguished from that of other salts. In this case the effect is negligible up to 3 per cent and then becomes pronounced. The results are given in table 3. Very likely the enhanced cohesion results from the slow formation of calcium silicate, in which case we are not dealing with the cohesion of the soil but with that of crystals of calcium silicate.

The effect of driving away the water of constitution on the cohesion of soils was also studied by heating single-base soils to various temperatures for 4

TABLE 2
Effect of CaCl_2 on the cohesion of sodium soil

CaCl_2 PER 100 GM. SOIL	COHESION
<i>m.e.</i>	<i>kgm.</i>
0	32.0
2	26.4
4	24.5
6	24.5
8	20.7
10	19.0

TABLE 3
Effect of freshly precipitated calcium silicate on soil cohesion

CALCIUM SILICATE	COHESION
<i>per cent</i>	<i>kgm.</i>
0	16.8
1	15.4
2	17.3
3	17.7
4	32.0
5	44.6

hours. The results, given in table 4, show that cohesion first decreases and then increases. The decrease is very likely due to the destruction of the colloidal surface, resulting in a certain amount of contraction and reduction in the points of contact. The initial dehydration is followed by the fusion of the silicates, resulting in an increase in cohesion. It is a remarkable fact that the cohesion of the dry soil is as great as that of the soil ignited at 800°C .

As glycerine and sugar are supposed to increase the ionization of acids (1), the cohesion of H-soil should increase in the presence of these substances. One part of glycerine or sugar was added to 100 parts of a H-soil brought to different pH values by the addition of NaOH. The cohesion of these soils was then determined in the dry state. The results, plotted in figure 5, show that,

except for the glycerine-treated soil at 11 pH, cohesion is slightly increased at all pH values. The effect of sugar at high pH value is extraordinarily great. It is interesting to note that the effect of glycerine and sugar is maximum at pH 7.5. An increase beyond this pH results in a reduction in the cohesion.

In view of the importance of molasses in the stabilization of earth roads, its effect on cohesion was studied in some detail. Increasing quantities of molasses were added to a Na- and a Ca-soil, and cohesion was measured after drying over H_2SO_4 . The results, given in table 5, show that both soils give increased cohesion with molasses. The maximum value is reached with 2 per cent molasses, and further increase apparently has no effect. The high cohesion values for the Na-soil as compared to the Ca-soil are noteworthy.

TABLE 4
Effect of heating soils at different temperatures on cohesion

NATURE OF CATION	COHESION						
	H_2SO_4 - dried	150°C.	300°C.	500°C.	600°C.	700°C.	800°C.
	kgm.	kgm.	kgm.	kgm.	kgm.	kgm.	kgm.
Na.....	24.2	14.0	11.8	14.6	19.1	19.1	24.0
K.....	16.0	10.0	7.4	8.2	9.6	9.6	13.0
Mg.....	11.6	6.4	4.1	5.4	6.0	6.0	10.0
Ca.....	8.2	5.0	3.6	4.1	5.0	5.0	7.3
H.....	5.4	4.0	3.2	3.2	3.7	4.1	5.4

TABLE 5
Effect of molasses on the cohesion of Na- and Ca-soils

MOLASSES..... per cent	COHESION			
	0	1	2	3
	kgm.	kgm.	kgm.	kgm.
Na-soil.....	23.6	28.0	43.2	41.5
Ca-soil.....	10.0	12.3	21.0	20.4

To determine whether the enhanced cohesion of the dry soil is lost on rewetting, the Na- and Ca-soils containing 2 per cent molasses were rewetted in atmospheres of increasing humidities, and the cohesion was determined after the pellets had reached a state of equilibrium with moisture. The results, given in table 6, show that cohesion decreases on rewetting.

To explain the binding forces between clay particles in a soil crumb, Russell (6) has put forward the hypothesis that the particles are held together by orientated molecules of a polar liquid. These polar molecules lie between negative charges on the clay surface and the exchangeable cations that have dissociated from the clay surface and are strongly orientated in the electrostatic field between these charges. The following objections to this hypothesis might be raised:

Soft crumbs are given only by those liquids in which the soil would not disperse, and without dispersion the colloidal clay which acts as binder cannot be released. For the forces of cohesion to come into play, the substance should be either in solution or in a fine state of subdivision in such a way that the residue left on evaporation is amorphous. It is for this reason that gum or glue behaves as an excellent binder. None of these substances would show any binding property if the liquid used did not act as a solvent or a dispersed medium. The important consideration is whether a substance can dissolve or disperse in a liquid and not whether the latter is polar or nonpolar. Certain resins dissolved in nonpolar liquids might yield excellent binding material on drying.

If the soil is completely dried, the binding link due to the orientated molecules of the dispersion medium must break down and the soil fall to a powder, but no such thing happens.

The soil colloids behave like weak electrolytes, and the proportion of cations dissociated is very small as compared to the total base (hardly 1 per cent). Any effect due to the orientation of dissociated ions, therefore, would be slight. Further, in accordance with the general behavior of weak electrolytes, the number of dissociated ions must decrease as water evaporates and the solution becomes more concentrated. The loss of moisture therefore must result in a decrease of cohesion, a conclusion contrary to fact.

In view of the enormous increase in cohesion when the last traces of water are removed from soil, any explanation based on the orientation of water

TABLE 6
Decrease in cohesion on rewetting of soil containing 2 per cent molasses

HUMIDITY.....per cent	COHESION					
	0	10	30	50	70	98
	kgm.	kgm.	kgm.	kgm.	kgm.	kgm.
Na-soil.....	38.6	38.10	28.2	25.4	16.4	6.4
Ca-soil.....	25.4	23.0	20.0	18.2	11.8	6.4
Na-soil with cement.....	18.2	25.4	16.3	14.5	12.3	6.0

molecules is untenable. Not only is the actual amount of dissociated ions in a soil small, but in the presence of a small quantity of an electrolyte (1) it becomes almost negligible, an amount which would produce no effect on cohesion. It would be a mistake to consider the soil surface as consisting only of dissociated ions. The titration curves of soil suspensions, the changes in conductivity due to dilution, and the rapidity of base-exchange reactions, all point to the fact that the system soil-water is like a homogeneous solution—homogeneous in the sense that the water phase under the equilibrium condition has the same concentration of dissolved cations throughout the entire mass, and in a given system a definite proportion of the cations are dissociated in accordance with the law of mass action.

The exact mechanism of the enormous increase in cohesive forces in soils on drying can be visualized by supposing that the minute interstices between the particles are filled not with water but with a suspension of colloidal clay, which binds the particles together on drying very much like a solution of gum. It has been shown in a previous paper (4) that the relation between moisture

content and relative humidity can be accounted for by the supposition that as the soil dries, the interstices between the larger particle are first emptied and the moisture gradually recedes into the interstices between smaller and smaller particles. The enormous increase in cohesion when the last traces of water are removed is easily understood. If we accept the hypothesis that colloidal clay acts like glue in binding the larger particles, it would follow that other colloidal substances like egg albumen, gum, rice starch, and even skim milk would enhance soil cohesion. The action of substances like sugar and molasses that leave an amorphous residue can also be visualized on a similar basis. In table 7 are recorded the cohesion values of a soil to which increasing amounts of colloidal substances have been added. The cohesion is more than doubled in the presence of 5 per cent egg albumen and more than trebled in the presence of a like percentage of rice starch and of 1 per cent gum. The effect of skim milk is not so pronounced.

TABLE 7
Effect of colloidal substances on the cohesion of soil

RICE STARCH		SKIM MILK		EGG ALBUMEN		GUM ARABIC	
Amount	Cohesion	Amount per 100 gm. soil	Cohesion	Amount	Cohesion	Amount	Cohesion
<i>per cent</i>	<i>kgm.</i>	<i>cc.</i>	<i>kgm.</i>	<i>per cent</i>	<i>kgm.</i>	<i>per cent</i>	<i>kgm.</i>
0	21.1	0	21.1	0	21.1	0.0	21.1
1	37.0	10	24.5	1	29.3	0.25	48.1
2	51.5	20	23.8	2	40.0	0.5	55.3
3	65.6	30	23.2	3	43.6	0.75	57.7
4	72.4	40	25.4	4	51.0	1.00	69.5
5	76.0	50	25.9	5	50.0	1.50	69.5

REVERSIBILITY OF COHESION

A problem of great practical importance is the reversibility of cohesion. As regards the relation between cohesion and hygroscopic moisture, the reversibility appears obvious. Beyond the slight hysteresis effect, the cohesion increases on drying and decreases on wetting. The point is of considerable importance in water-stabilized earth roads. The effect of remaking the semi-spherical pellets after breaking them was studied with single-base soils, in both the dispersed and the undispersed state. The results given in table 1 show that there is virtually no difference in cohesion between the original pellet and the pellet that is powdered and remade into the same shape for test. The difference between the cohesion values for dispersed and undispersed soil has already been referred to, and it is noteworthy that the dispersed soils maintain these higher values on remaking. It must be emphasized that the nature of the replaceable base is important only indirectly, insofar as it is responsible for determining the state of dispersion of the soil colloids.

Another aspect of the reversibility of soil cohesion which refers to stabilized soils and which is even more important from the practical point of view is the question of how far it is possible to restore the cohesion of a soil with water alone after its mechanical breakdown. Obviously substances that are normally insoluble in water and are applied in the form of an emulsion cannot be expected to fall under this category. Soluble substances like molasses, on the other hand, could possibly be brought to the same state of subdivision and intimate mixture with the soil, and original cohesion restored after a mechanical breakdown. The possibility is not remote, however, that molasses might become oxidized and disappear partly or wholly in course of time. The irreversibility of soil colloids other than those containing exchangeable sodium would also be an important factor.

Pellets of a soil stabilized with molasses were broken and remade several times, cohesion being recorded each time. It will be seen from table 8 that the high values of cohesion with molasses are maintained when the pellet is broken and remade. The possibility that repeated alternate wetting and

TABLE 8

Cohesion of soil stabilized with molasses, as affected by breaking and remaking, wetting and drying

ORIGINAL WITHOUT MOLASSES	WITH MOLASSES	
	Breaking and remaking	Wetting and drying
kgm.	kgm.	kgm.
21.2	50.0	50.0
....	58.2	53.0
....	47.0	50.5
....	53.6	55.7
....	48.2	51.5

drying might lead to a deterioration of the cohesive bond imparted by molasses was also studied. Soil stabilized with molasses was subjected to alternate drying and wetting by storing over 10-90 per cent humidities, cohesion being measured after a definite number of cycles. The results given in table 8 indicate that alternate drying and wetting does not alter the cohesive forces.

SUMMARY

Cohesion for the same percentage of clay rapidly falls as the particle size increases up to a limit beyond which the effect of size of particles becomes negligible.

In single-base soils, the order of cohesion for the dry soil follows the generally accepted order of dissociation for these ions.

REFERENCES

- (1) CORRAN, J. W., AND LEWIS, W. C. M. 1922 Effect of sucrose on the activities of the chloride and hydrogen ions. *Jour. Amer. Chem. Soc.* 44: 1673-1684.

- (2) PURI, A. N. 1935 A siltometer for studying size distribution of silts and sands. *Punjab Irrig. Res. Inst. Res. Pub.* 2 (7).
- (3) PURI, A. N. 1937 Physical characteristics of soils: I. New methods of measurements. *Soil Sci.* 44: 481-487.
- (4) PURI, A. N. 1939 Physical characteristics of soils: V. The capillary tube hypothesis of soil moisture. *Soil Sci.* 48: 505-520.
- (5) PURI, A. N., PURI, B. R., AND LAL, M. 1938 Dispersion and stability of soil colloids in water: II. Ultra clay and the efficiency of dispersion methods. *Punjab Irrig. Res. Inst. Res. Pub.* 4 (11).
- (6) RUSSELL, E. W. 1935 The binding forces between clay particles in a soil crumb. *Trans. Third Internatl. Cong. Soil Sci.* 1: 26-29.



ALEXIUS A. J. DE' SIGMOND

Photo by Erdélyi, Budapest

Alexius A. J. de' Sigmond

1873-1939

It is with great regret that SOIL SCIENCE records the death, on September 30, 1939, of one of its consulting editors, Dr. Alexius A. J. de 'Sigmond, professor of agricultural chemistry and technology and of soil science in the Royal Hungarian Palatine-Joseph University of Technical and Economic Sciences of Budapest, and formerly director of the Royal Hungarian Institute of Chemistry and Central Experimental Station at Budapest.

Prof. de 'Sigmond was born in 1873 at Kolozsvár, Hungary, the son of an industrialist and member of an old aristocratic Transylvanian family. He studied chemistry at the Technological Institut in Vienna, graduating with the degree of chemical engineer in 1895. During his years at the university, he became interested in agricultural science. He carried out his first investigation in his father's brewery and alcohol distillery, on "The action of diastase upon raw starch." His doctor's dissertation dealt with "The reaction velocity of maltose hydrolysis." He received his doctor's degree in 1898 from the University of Kolozsvár. In 1899, he left his father's plant and became assistant chemist at the Agricultural Experiment Station at Magyaróvár, where his interest in soil problems was stimulated by his chief, Alexander Cserhati.

His work at Magyaróvár resulted in a series of papers published in the Hungarian, German, French, and English languages in various scientific journals. These papers dealt with a number of problems, chief among which was the determination of assimilable nutrients in the soil.

In 1905, he became privat docent in agricultural chemistry at the University of Budapest, and professor at the Technological Institut, where he was entrusted with the organization of the Institut of Agricultural Chemistry. This was soon followed by a scientific trip abroad, where he visited various agricultural institutes, including those in the United States. Here he had an opportunity to familiarize himself with the work of the famous American soil chemist, Prof. E. W. Hilgard, at the University of California, which had a great influence upon his subsequent work.

In addition to his teaching activities, Prof. de 'Sigmond carried out extensive investigations in the field of agricultural industry and soil science. He devoted particular attention to the question of alkali soils. His method of determination of assimilable phosphoric acid in the soil, his efforts to improve alkali soils in Hungary, and his attempts to apply to agricultural practice results obtained in theoretical investigations aroused much interest and found extensive

application. In addition to numerous popular contributions published in the Hungarian language, he wrote two large works, "The Principles of Soil Science" and "The Hungarian Alkali Soils and Methods of Their Reclamation," both of which were translated into English and published, the first in London and the second at the University of California.

In 1910, he was elected president of the Soil Science Conference held at Stockholm. He organized the International Commission for Soil Chemists, which met first in 1914 in Munich, and again in 1922 in Prague. When the International Soil Science Society took form in Rome, in 1924, his commission was bodily incorporated as Commission II, of which he was made president. In 1935, at the Soil Science Congress at Oxford, he was made honorary president of the commission and honorary member of the society.

For his scientific activities he was variously honored in his own country. He was made a member of the Hungarian Academy of Sciences and of the St. Stephen Academy, chairman of the Hungarian Agricultural Experiment Station, and president of the Hungarian Commission for Soil Improvement. He received various honors also from his government.

His untiring efforts were directed toward the study of the soil, its chemical composition and classification, and especially the reclamation of semiarid soils.

Prof. de 'Sigmond was an artist by nature and a brilliant pianist. Those soil scientists who participated in the American Excursion following the First Congress held in Washington, in 1927, will remember his delightful improvisations. He had great personal charm and was a brilliant conversationalist. His death is a great loss to soil science.

S. A. WAKSMAN

HYDROLYSIS OF UREA IN SOILS BY THERMOLABILE CATALYSIS¹

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That several kinds of bacteria can transform urea to ammonium carbonate in soils has been amply demonstrated. No other concept has seemed necessary to explain most existing data. In consequence, it has generally been assumed that these microorganisms are the chief agents in soils directly responsible for this change. Conrad and Adams (2), however, obtained data which suggest that the reaction may, in part, be catalytic rather than completely biological. In their experiments, untreated and heavily tolued soil showed by subsequent plant cultures no significant difference in the definite ability of the soil to remove the nitrogen from a percolating solution of urea. Preheating of the soil largely destroyed this ability. Reporting confirmatory laboratory studies, this paper presents further evidence that the hydrolysis of urea in the soil examined, Yolo fine sandy loam, was mostly catalytic rather than directly microbial.

LABORATORY STUDIES OF PERCOLATION

With the growth studies previously described (2), the mechanism of the transformations and the various factors influencing them were not always clear. In view of the general nitrogen deficiency of the soil, the enhanced growth indicated solely the location and relative amounts of the added nitrogen but nothing of its form or of the transformations that had taken place during percolation. A laboratory study of the changes in solutions of urea resulting from various rates of percolation, concentrations of urea, and soil treatments should throw some light on the mechanisms involved. The data reported in table 1 were obtained from percolations made in duplicate and at laboratory temperatures somewhat lower than average. One kilogram of dry soil was placed in each of four glass percolators. For the treatment under toluene, the dry soil was added to each percolator in three successive portions, about 10 cc. of toluene being added after each portion. Then, 20 cc. of toluene was mixed with a liter of the urea solution to be percolated through the soil. The drainage from the percolator dripped into a flask, the mouth of which fitted closely to the bottom of the percolator. Initially,

¹ Contribution from the Division of Agronomy, University of California, Davis.

² Many of the analyses reported herein were kindly carried out by R. E. Malde and J. M. Weiler, technicians in the Division of Agronomy.

330 cc. of urea solution was added to wet the soil. Then, 8 hours later and approximately every 12 hours thereafter, 100 cc. of the solution was added to the top of the percolator. Each percolate was transferred from the receiving flask just before each new increment of solution was added to the top of the percolator. In the toluene tests an odor of this antiseptic was always evident in the receiving flask as well as at the top of the percolator where a few milliliters of the toluene collected toward the end as an oily-appearing film. The residual urea was determined by Marshall's urease method, essentially as described by Hawk and Bergeim (7, p. 712). The data reported in table 1 show very little difference in the concentration of urea between the respective percolates from the two treatments.

In adding the increments of urea solution to the percolators, the volume of toluene was not considered. Undoubtedly, if equal volumes of solution had been collected in the first percolates and in the subsequent ones, the small

TABLE 1

Concentration of urea in successive 100-cc. percolates from normal soil and from soil under toluene—Yolo fine sandy loam

Results in milligram atoms of N per liter

SOIL TREATMENT	CONCENTRATION OF UREA IN							
	Original solution	Successive percolates						
		1	2	3	4	5	6	7
None.....	39.0	5.6*	21.4	32.0	32.6	32.6	33.2	34.0
Under toluene.....	39.0	7.6†	24.8	34.0	33.4	33.6	33.4	33.8

* Only 25 cc. collected.

† Only 45 cc. collected.

differences between the successive percolates of the two treatments would have been even smaller.

The great reduction in concentration in the first and second percolates is clearly assignable to adsorption of urea by the soil solids. This phenomenon will be given further consideration.

The results in table 1 challenge the prevailing popular conception that the decomposition of urea in soils is mainly accomplished by soil microorganisms. Catalytic hydrolysis of urea in soils by some mechanism not involving living organisms directly is indicated by these data, which showed nearly equal reduction in the concentration of the percolating urea solutions regardless of the presence or absence of the antiseptic, toluene. After the rather small adsorptive capacity of the soil was taken care of and dynamic equilibrium was established between the soil and the percolating solution, moving a solution of urea at a constant rate under a constant environment through a soil with an effective catalyst of constant though low activity should result in a constant rate of withdrawal of nitrogen from solution, at least until secondary effects set in.

Subsequent trials by this or similar procedures were generally made as single determinations with 400 gm. of dry soil in 4-inch clay pots previously covered with asphaltum paint. A square of waxed paper was placed over the drainage hole, and the dry soil was added. The pot was then nested into the drainage can or into a glass beaker or other receptacle of appropriate size. The soil was wet initially with 130 cc. of the urea solution, after which 75-cc. increments were added periodically. The few milliliters that drained out from the 130 cc. initially applied are referred to as "drippings" or as "drip." In addition to the untreated, soil treated as follows was studied: (a) moistened soil, preheated for 48 hours to about 85°C., and subsequently dried as described previously (2); (b) soil preheated to about 105°C. The moistened soil was placed in an oven at 120°C., allowed to dry, and remoistened and dried several times. Undoubtedly, the soil itself, while moist, did not attain a temperature above 105°.

The actual concentrations of urea in the percolates are in themselves of interest from many points of view. But from the standpoint of urea-adsorbing and urea-splitting powers of the soil, the reductions in urea concentrations below those in the original solutions are of much more interest. In consequence, these reductions for successive percolates have been calculated and are shown graphically in figure 1.

The data plotted on the left indicate that with soil preheated to 85°, considerable urea was removed from solution in the drippings and in the first percolate. From the second to the fourth percolates the concentrations of the urea solutions were but very little different from the original concentrations, regardless of what the actual concentrations were. The type of behavior there exemplified would be expected if the urea were adsorbed on the soil colloids.

In investigations with the normal soil, the concentration of urea first coming through was much reduced below the original concentration, and as successive percolates were collected the concentration rose and then levelled off at a concentration somewhat below the original. Where the solution was percolated through rapidly, the amount of urea removed from solution was smaller. As the time interval between the additions was lengthened, the amount of urea removed from solution increased. These reductions are shown graphically on the right in figure 1. The same types of curves would be obtained if the data of table 1 were similarly plotted. The amount of urea removed from solution after the concentration had become very steady was almost the same regardless of its original concentration. Thus, for 12-hour intervals between successive increments, almost the same amount of urea was removed from a concentration of 104.8 m. at. ³N per liter as from one of 26.1. As the time interval was increased to 48 hours, the amount of urea removed was considerably increased. Varying the concentration of the urea solution has, thus, had very little effect upon the rate of hydrolysis. This phenomenon

³ As used in this paper, m. at. indicates milligram atoms.

is typical of the action of certain enzymes (3, p. 154) including urease (14). Again with hydrolysis at least roughly proportional to the time of the contact of the solution and the soil, a catalytic rather than a microbial process was indicated.

Data similar to those for Yolo fine sandy loam have been obtained with several other soils. But as studies on these soils have been extended to include additional factors outside the scope of this paper, publication of these data will be deferred.

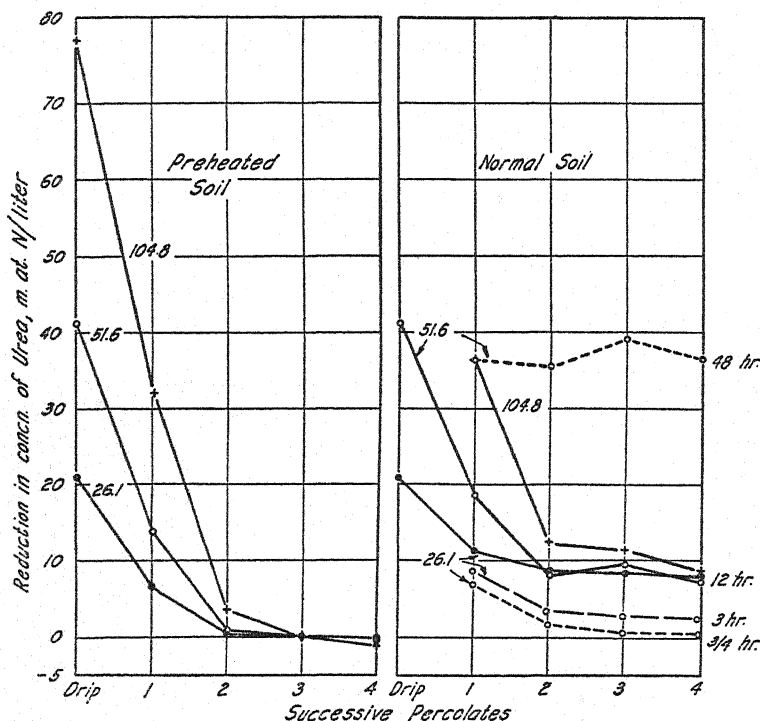


FIG. 1. REDUCTION IN THE CONCENTRATION OF UREA SOLUTIONS PERCOLATED THROUGH 400 GM. OF PREHEATED AND NORMAL YOLO FINE SANDY LOAM

The numbers at the left of each curve indicate the concentration in milligram atoms of nitrogen per liter; those at the right, the interval between successive 75-cc. additions to the soil.

In order more closely to correlate these laboratory studies and the greenhouse ones reported previously (2), additional percolation tests were made. These are reported in table 2. In addition, soil preheated to 105°C. was treated by mixing 0.02 gm. of Jack-bean urease with 300 gm. of soil and adding this to a pot which contained 100 gm. of the preheated soil. It will be observed that the urease caused almost complete removal of the urea from the percolating solution.

The replicated growth-percolation studies reported in table 3 and the analy-

ses in table 4 of a previous paper (2) became clearer in the light of these laboratory data. The low concentrations of urea in the percolates reflect largely the withdrawals by adsorption. The still lower concentrations by slow percolation are, for the untreated and tolunened soils, attributable to catalysis and less volume of solution (cf. percolate 1, table 1 and drip, fig. 1) and, for the preheated soil, to less volume and possibly to some small amount of thermostable catalysis, further indications of which will appear below. The data of tables 1 and 2, and of figure 1 of this paper indicate that adsorption was an important factor in reducing the concentration of the solution reaching the bottom pots in the former study. As was to be expected, the rate of percolation had but little effect upon the nitrogen retained in the pots containing the preheated soil. With untreated and tolunened soils, however, the

TABLE 2

Effect of different factors upon the change in concentration of a urea solution passing through 400 gm. of Yolo fine sandy loam in a 4-inch pot as determined in successive 75-cc. percolates

Results in milligram atoms of N per liter

SOIL TREATMENT	CONCENTRATION OF UREA IN				
	Original solution	Successive percolates*			
		1	2	3	4
None.....	0.0	0.2	0.2	0.0	0.0
None.....	20.0	8.6	10.6	11.2	11.2
Preheated to about 85°C.....	20.0	12.8	18.6	19.4	19.0
Preheated to about 105°C.....	20.0	lost	18.8	19.2	18.4
Preheated to about 105°C. and mixed with 0.02 gm. urease.....	20.0	2.4	0.4	1.2	1.0

* The time interval between collections of successive percolates of the urea solutions was 12 hours.

slower rate of percolation by allowing more time for catalysis had much effect in retaining the nitrogen of urea.

ADSORPTION OF UREA

The question arises whether the preheating has in any way changed the adsorption capacity of the soil. The more rapid the movement of the urea solution through normal soil, the more nearly catalytic hydrolysis was reduced to a minimum as measured by reduction in concentration of the solution. In the addition of urea at $\frac{3}{4}$ -hour intervals, most of the nitrogen removed would be due to the adsorption of urea. The shape of the curves in figure 1 are in full agreement with this conception. The curves for 26.1 m. at. N per liter percolated at $\frac{3}{4}$ -hour intervals through normal soil and at 12-hour intervals through preheated soil are almost identical. If from this comparison the inference is drawn that very little or no change in the adsorptive capacity of the soil for urea has been brought about by preheating as done in this study,

soil so treated, then, seems to be very suitable material for the study of adsorption itself, with minimum danger of any vitiating catalysis being encountered. In the equilibrium method (6) used, 100 gm. of the preheated soil (containing 0.85 per cent hygroscopic moisture) and 150 cc. of the urea solution were intimately mixed, shaken periodically for 15 hours, and then filtered. From the analysis of the urea in the filtrate, computations were made of the amount adsorbed by the soil. These data are plotted logarithmically in figure 2. A straight line comes close to the experimental points. This would indicate that a true adsorption was the factor largely responsible for the removal of urea from solution by preheated soil.

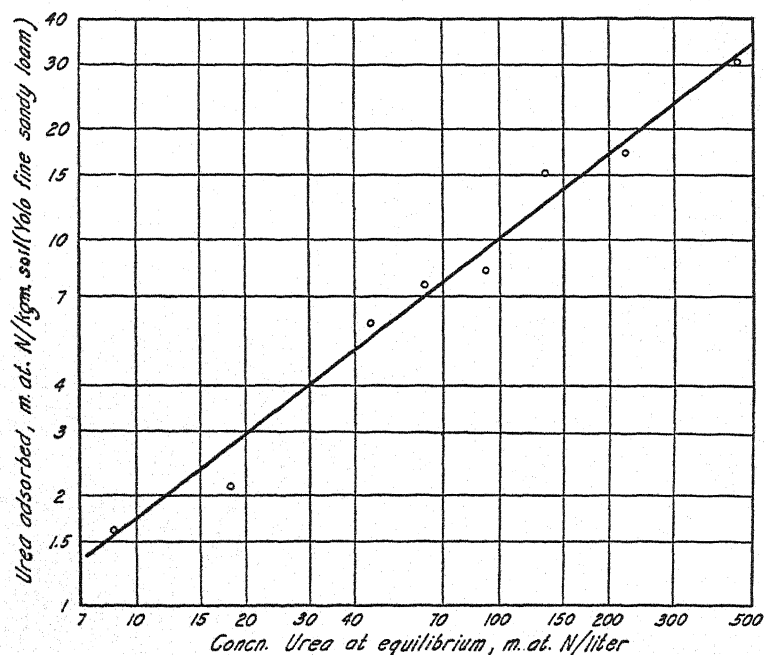


FIG. 2. ADSORPTION OF UREA BY YOLO FINE SANDY LOAM

In another method used to study adsorption, a 200-gm. portion of preheated soil was placed in a glass percolator and percolated with 300 cc. of the urea solution. The percolate was collected as one sample and analyzed for urea. In this way the amount of urea remaining in the soil was computed. From the difference in the amount of solution, on the basis of the original concentration of the urea, the amount of urea remaining in solution in the soil was next computed. The urea computed, then, to be adsorbed on the soil was taken as the difference. Two different concentrations were used as reported in table 3. Values calculated from figure 2 for the urea adsorbed by 198.3 gm. soil at the equilibrium concentrations involved in table 3, 2.62 m. at. N for test I and 6.6 for test II, are of the same magnitude as those in table 3.

After the urea solution had been completely percolated through the soil, the latter was successively leached with 150-cc. portions of distilled water. The leachates were collected in order, analyzed, and the absolute amount of urea removed was calculated. The first leachate in each case removed more urea than was calculated to be present in the soil in solution. The second and, in one case, the third leachate removed additional urea. The total amount of urea removed from the soil was sufficiently greater than the actual amount dissolved in the soil solution to account for more than half of the adsorbed urea. This reversibility of adsorption of urea by the soil is one criterion of true adsorption.

TABLE 3

Reversibility of urea adsorption by preheated Yolo fine sandy loam

Urea adsorbed from solution by preheated soil was in part removed by subsequent leaching

UREA	TEST I		TEST II	
	cc.	m.at.N	cc.	m.at.N
Original solution.....	300	43.78	300	143.8
Collected as percolate.....	<u>222.5</u>	<u>30.17</u>	<u>221.5</u>	<u>99.5</u>
Original remaining in soil.....	77.5		78.5	
Hygroscopic moisture 0.85 per cent.....	<u>1.7</u>		<u>1.7</u>	
	79.2	13.61	80.2	44.3
Remaining in soil solution.....		<u>11.56</u>		<u>38.4</u>
Adsorbed by 198.3 gm. soil.....		2.05		5.9
Recovered in:				
First leachate.....	150.5	12.31	149.5	40.75
Second leachate.....	58.3*	0.43	149.0	2.53
Third leachate.....			143.0	0.13
Total.....		<u>12.74</u>		<u>43.41</u>
Adsorbed urea removed by leaching.....		1.18		5.01

* Stopped filtering.

CATALYSIS

The evidence in the laboratory percolation studies suggested that the catalytic hydrolysis of urea had been the primary cause of the reduction in concentration of a urea solution passing through the soil. Certain evidence with regard to the effects of preheating, of varying the concentrations of urea, and of varying the rate of percolation points to catalysis as being involved.

The criterion of catalysis has been the steady reduction in concentration of a urea solution percolating through the soil. Another criterion of catalysis, the appearance of ammonia (or ammonium ion), was examined in incubation studies. For each test 75 gm. of soil was put in a covered glass jar, and 20 cc.

of urea containing 2 m. at. N was then added. Some of the soil was normal and some preheated. In one trial the preheated soil was inoculated with 0.5 gm. of normal soil for each 400 gm. of preheated soil. These were incubated for various lengths of time at 30°C. The results are reported in table 4. In some of these tests toluene was added, and in others it was omitted. For experiments 1 and 3 a lot of the same soil recently cropped to oats in the greenhouse to reduce nitrates to a low level was used. Soil like that used in the other studies reported herein was used in experiment 2.

TABLE 4
Effect of various soil treatments upon ammonia formed from urea during incubation

SOIL TREATMENT		MILLIGRAM ATOMS OF NITROGEN PER KILOGRAM SOIL AS AMMONIA FORMED DURING INCUBATION		AGENTS LARGELY RESPONSIBLE
Pretreatment	Antiseptic	3 days	7 days	
Experiment 1*				
Preheated.....	Toluene	0.27	0.40	Neither Microorganisms
	None	0.9	3.2	
None (normal).....	Toluene	10.6	8.0	Catalyst Both
	None	9.6	10.8	
Experiment 2				
Preheated.....	Toluene	0.67	0.80	Neither Microorganisms
	None	1.73	3.3	
None (normal).....	Toluene	7.2†	14.5†	Catalyst Both
	None	9.5†	15.5‡	
Experiment 3				
Preheated and inocu- lated§.....	Toluene	0.27	0.67	Neither Microorganisms
	None	2.1	2.8	

* Controls with distilled water showed only negligible amounts of ammonia formed. Urea was added at the rate of 26.7 m.at.N per kilogram of soil.

† Nitrates—a trace by diphenylamine test.

‡ Nitrates—strong.

§ Inoculated with 0.5 gm. normal soil per 400 gm. preheated soil.

Undoubtedly, many factors influence the rate of urea decomposition in the soil. No effort has been made in these studies to evaluate them fully, but an effort has been made to get a relative measure of the effects of the two agents splitting urea—microorganisms and the thermolabile catalyst. As shown in table 4 only small amounts of ammonia were produced in preheated soil under toluene with both agents inactivated. In the preheated soil not under toluene and therefore with catalyst inactive and microorganisms active, appreciably greater amounts of urea were hydrolyzed. In normal soil under toluene and therefore with microorganisms inactive and the soil catalyst

active, much more ammonia was formed. In these experiments as well as in those reported in tables 1 and 2, the thermolabile catalytic agent was much more active than microorganisms in splitting urea. It might be presumed that preheating would kill, among others, the types of organisms which could work under toluene, causing the changes reported in table 4. If that were the case, reinoculating the preheated soil with small amounts of the normal soil would seem to be adequate to restore all strains of microorganisms originally present. The amount of ammonia formed with this preheated and inoculated soil not under toluene was but little different from that formed where this soil was not inoculated at all.

DISCUSSION

The discovery of catalytic hydrolysis of urea in our soil naturally raises the question whether the disappearance of urea or the appearance of ammonia following the addition of urea in any untreated soil may not be due to catalytic hydrolysis rather than to bacterial activity. Gibson (4, 5) found that each of several soils caused the disappearance of about 1 per cent of its weight of urea in 24 hours. He attributed this loss of water-soluble urea to the activity of microorganisms directly. In the light of the present study a small part might have been removed from solution through adsorption by the soil, but the larger part must have been changed (presumably to ammonium carbonate) by hydrolysis, and the transformations might have taken place, and undoubtedly did take place, largely catalytically rather than microbially. Again, Kleberger (8), in a summary of his work, reported that natural differences in the ability of the soils studied to hydrolyze urea were destroyed by heating to 300°C. or by boiling in 2 per cent HCl, but were not destroyed by subjecting the soils to the antiseptic, chloroform.

Urea can be hydrolyzed by acids and alkalies (11). Preheating may change the pH of the soil, but such change cannot be advanced as the cause of the change in catalytic activity following this treatment, as the greatest activity took place with the untreated soil, which was approximately neutral.

On the assumption that the toluene applied to the dry soil inactivated or greatly inhibited the soil microorganisms decomposing urea (cf. exp. 3, table 4), the urea transformations in heavily tolued soil may be attributed to catalysis. The catalyst, it is true, may be an enzyme arising from previous microbial activity. As the toluene was added to the dry soil before, or at the same time as, the urea solutions, transformations could not be caused by the microorganisms directly. The transformations in the untreated soil were but little, if any, greater than those in the same soil heavily tolued. Assuming that these effects were additive, at least initially, the writer believes that catalysis (probably by an enzyme) was chiefly responsible for the urea transformations in the untreated soil.

The catalyst responsible seems to evince the properties of an enzyme (perhaps adsorbed on the soil colloids) better than any other known catalyst for

urea: catalysis went on in spite of toluene, was for practical purposes completely inactivated by preheating the moistened soil to 85°C., was independent of the concentration of urea, and was substantially proportional to the time of contact of urea solution and the soil.

Mention should be made of the discovery by Subrahmanyam (12) of deaminase activity in water-logged soils. He was able to elute this activity (13) from the soil, at least partially to purify it, and finally to show its activity outside the soil in the deaminization of glycine to ammonia and glycolic acid. Investigations are in progress designed to find out more about the catalyst evidenced in this present study.

In the study of the partial sterilization of soil by antiseptics, data (10) were accumulated which showed ammonification by catalysis. This has been held as evidence of enzyme activity (9, p. 366). These fragmentary bits of evidence, together with the data of the present study, may very well raise the question whether many of the other transformations in soils which have been attributed to microorganisms and which can, it is true, be carried on by them, may not in fact actually result from catalytic activity directly. More systematic studies, perhaps similar to the present one, will be necessary before this question can be answered.

SUMMARY

Laboratory studies were made of the changes in various urea solutions resulting from percolation through Yolo fine sandy loam. In studies with preheated soil, marked reductions in various concentrations of urea solutions were noted in the first percolates and less in the second ones. These reductions are best explained on the basis of adsorption of the urea by the soil. Later percolates were nearly the same in concentration as the respective original solutions.

In comparative studies with untreated soil and with soil under toluene, reductions in concentration likewise occurred with the first percolates, but the reductions in successively later ones leveled off and became nearly constant. These constant reductions with a constant rate of percolation are assignable to catalytic activity in the soil. This catalysis was thermolabile because it was inactivated by preheating.

Wide variations in the concentration of urea percolated through untreated soil had very little effect on this catalytic activity as measured by reductions in the concentration of urea. This phenomenon suggests that the activity of an enzyme was involved. The amount of the reduction in concentration was substantially proportional to the length of time between the additions of successive amounts of the urea solutions.

Equilibrium trials between various concentrations of urea and soil preheated (and therefore devoid of the thermolabile catalytic activity) were conducted to study adsorption. The amounts adsorbed plotted logarithmically against the equilibrium concentrations gave points very close to a straight

line, thus indicating true adsorption. In percolation studies urea adsorbed by preheated soil was substantially recovered by subsequent leaching with water.

In incubation trials preheated soil under toluene produced but little ammonia from urea; preheated soil without toluene produced somewhat more; normal soil under toluene produced much more, almost as much as normal soil without toluene. Reinoculating the preheated soil with about 0.1 per cent of normal soil had but little effect on the amount of ammonia formed.

These experiments indicate that in the soil studied, the hydrolysis of urea resulted much more from catalysis than from the direct simultaneous action of the soil microorganisms themselves.

It is possible that the hydrolysis of urea in untreated soils in general may be mainly catalytic rather than entirely directly microbial, as experiments attributing the activity to microorganisms have not been designed to test the possibility of catalysis. The question whether other organic transformations in untreated soils may be, in part at least, catalytic rather than entirely microbial is raised by the present experiments.

REFERENCES

- (1) CONRAD, J. P., AND ADAMS, C. N. 1939 Determining by plant response the retention of nutrient ions by soils. *Jour. Amer. Soc. Agron.* 31: 29-34.
- (2) CONRAD, J. P., AND ADAMS, C. N. 1940 Retention by soils of the nitrogen of urea and some related phenomena. *Jour. Amer. Soc. Agron.* 32: 48-54.
- (3) FALK, K. G. 1924 *The Chemistry of Enzyme Actions*, ed. 2. Chemical Catalog Co., New York.
- (4) GIBSON, T. 1930 Decomposition of urea in soils. *Jour. Agr. Sci.* 20: 549-58.
- (5) GIBSON, T. 1930 Factors influencing the decomposition of urea in soils. *Zentbl. Bakt.* (II) 81: 45-60.
- (6) GORTNER, R. A. 1938 *Outlines of Biochemistry*, ed. 2. John Wiley & Sons, New York.
- (7) HAWK, P. B., AND BERGEIM, O. 1937 *Practical Physiological Chemistry*, ed. 11. P. Blakiston's Son & Co., Philadelphia.
- (8) KLEBERGER. 1920 Über den Verlauf der Harnstoffumsetzung in Sandkulturen und in Boden. *Landw. Vers. Sta.* 107: 298-301.
- (9) RUSSELL, E. J. 1937 *Soil Conditions and Plant Growth*, ed. 7. Longmans, Green & Co., London.
- (10) RUSSELL, E. J., AND HUTCHINSON, H. B. 1909 The effect of partial sterilization of soil on the production of plant food. *Jour. Agr. Sci.* 3: 111-144.
- (11) SIDGWICK, N. V. 1937 *The Organic Chemistry of Nitrogen*. (New ed., revised and rewritten by T. W. J. Taylor and Wilson Baker.) Clarendon Press, Oxford.
- (12) SUBRAHMANYAN, V. 1927 Biochemistry of water-logged soils: I. The effect of water-logging on the different forms of nitrogen, on the reaction, on the gaseous relationships, and on the bacterial flora. *Jour. Agr. Sci.* 17: 429-448.
- (13) SUBRAHMANYAN, V. 1927 Biochemistry of water-logged soils: II. The presence of a deaminase in water-logged soils and its role in the production of ammonia. *Jour. Agr. Sci.* 17: 449-467.
- (14) VAN SLYKE, D. D., AND CULLEN, G. E. 1914 The mode of action of urease and of enzymes in general. *Jour. Biol. Chem.* 19: 140-180.

THE ROLE OF ALGAE IN THE NITROGEN CYCLE OF THE SOIL¹

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The role of microorganisms in the fixation of atmospheric nitrogen and the factors which influence this phenomenon have been extensively studied by microbiologists for many years and are still the subject of much research. Although by far the greatest amount of work has dealt with the nitrogen-fixing bacteria, much interest and controversy have centered about the possible functions of algae in the fixation process. At the present time, it is generally believed that algae take part in the cycle of nitrogen in soil in two distinct ways: directly, by fixing gaseous nitrogen; and indirectly, by supplying nitrogen-fixing bacteria, particularly *Azotobacter*, with available, photosynthetically produced carbon compounds as sources of the energy necessary for the fixation process. Within recent years, it has been definitely established that certain species of blue-green algae can fix atmospheric nitrogen, although their practical importance in the nitrogen economy of the soil remains to be determined, since very little information is available concerning the distribution and abundance of these algae in soils and the conditions under which fixation will occur naturally. The second function has been assigned to algae almost from the beginning of their study as nitrogen-fixing agents and is regarded as commonly accepted. Even this role of algae, however, is largely hypothetical and is unsupported by convincing experimental evidence.

In the present investigation, an attempt has been made to test the indirect role of algae in nitrogen fixation by means of carefully controlled experiments. Some data will also be presented concerning the nitrogen-fixing ability of blue-green algae and their distribution in various soils.

RELATION OF AZOTOBACTER TO CHLOROPHYCEAE

Frank's contention in 1889 (12), based on impure culture work, that all algae possess the ability of fixing atmospheric nitrogen was soon challenged. Kossowitsch (16) isolated a grass-green alga, *Cystococcus*, in pure culture and showed that it could not fix free nitrogen. When soil suspensions were inoculated into mineral salt solutions and incubated in the light, however, algal development occurred and nitrogen was fixed; the fixation was most marked in those flasks in which blue-green algae, especially *Nostoc*, developed. Kosso-

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witsch concluded that algae, though unable to fix nitrogen, may aid indirectly in the nitrogen-fixing process by supplying carbohydrates to the nitrogen-fixing bacteria; he compared this process to the symbiosis existing between nodule bacteria and leguminous plants. Schloesing and Laurent (23) had previously hinted at this possible relationship between algae and nitrogen-fixing bacteria, but Kossowitsch was the first to advance this idea in a concrete form. His work, however, was open to the same criticism which he, himself, directed against Frank; that is, he failed to determine the effects of pure cultures of algae on pure cultures of nitrogen-fixing bacteria. In recent years, some species of blue-green algae; namely, *Nostoc punctiforme*, *Anabaena variabilis* (8), *Nostoc muscorum* (2), *Anabaena gelatinosa*, and *Anabaena naviculoides* (7), have been shown to be capable of fixing nitrogen. The increase in nitrogen found by Kossowitsch in his cultures may have been due to the activity of blue-green algae rather than to their symbiosis with bacteria.

The idea of a mutually beneficial interrelationship between algae and nitrogen-fixing bacteria, or at least one beneficial to the bacteria, has persisted from the time of Kossowitsch down to the present and can be found, expressed or implied, in the work of numerous investigators (4, 10, 13, 14, 18). More specifically, Reinke (21) found *Azotobacter* on balls of *Volvox* obtained from pond water; Bouilhac and Giustiniani (5, 6) inoculated sand, freed of organic matter, with *Cyanophyceae* (impure cultures) together with a small amount of soil suspension. They were able to grow buckwheat, mustard, corn, or cress in the sand under these conditions. Chemical analyses showed that an actual increase in nitrogen had occurred. As in the work of Kossowitsch, it is impossible to decide whether the algae or the bacteria fixed the nitrogen. Hugo Fischer (11) isolated *Azotobacter* from dark green *Oscillatoria* colonies. Nakano (20) found more nitrogen in flasks containing pure cultures of algae and *Azotobacter*, after an incubation period of a few months, than in those flasks which contained only *Azotobacter*. He could not explain these results. The action of the algae in this case may have been due to the conservation of the nitrogen fixed by the *Azotobacter*, i.e., by utilizing the NH_3 liberated during the autolysis of the bacterial cells and converting it into the organic nitrogen of the algal cell, thus preventing its loss to the atmosphere. This seems probable because algal development did not occur until the *Azotobacter* cells had passed their active stage and had begun to autolyze. Emerson (9) found that the nitrogen-fixing ability of *Az. vinelandii* and *Az. chroococcum* was stimulated when these organisms were grown on agar in the presence of algae (impure cultures). Lipman and Teakle (19) obtained nitrogen fixation by growing pure cultures of *Chlorella* and *Az. chroococcum* together in an inorganic medium to which no carbohydrate had been added. The amount of nitrogen fixed in 50 cc. of culture medium, however, was only about 0.5 mgm. Jones (15) found aerobic and anaerobic nitrogen-fixing bacteria in the mucous sheaths of *Nostoc*, *Rivularia*, and *Gloecapsa*. On the other hand, Allison and Morris (3) reported that there was no increase in nitrogen fixation by *Az. vinelandii* when

grown in the presence of pure cultures of *Chlorella*, *Chlamydomonas*, *Scenedesmus*, and *Pleurococcus* but presented no data to prove this statement.

Although the concept of a beneficial symbiosis between algae and nitrogen-fixing bacteria, particularly *Azotobacter*, is generally accepted, the review of the literature appears to indicate that the experimental results on which this conception is based are open to one or more of the following criticisms:

The investigations were made with impure cultures, and therefore the experimental conditions were inadequately controlled.

In most of the impure culture work, nitrogen fixation was found only in those cases where there was an abundant growth of blue-green algae; these may have been directly responsible for nitrogen fixation rather than indirectly, by supplying carbohydrates to the nitrogen-fixing bacteria.

In the one instance where pure cultures were used, the amounts of nitrogen reported to have been fixed were small enough to be within the experimental error of the nitrogen determination.

It was with these facts in mind that the following experiments were performed in an attempt to determine by pure culture methods whether algae could supply *Azotobacter* with available energy and carbon compounds.

Various pure cultures of *Chlorophyceae* were grown together with *Azotobacter* in a mineral salt medium which contained fixed nitrogen, but to which no organic compound had been added. This medium is suitable for the growth of the algae. The *Azotobacter*, on the other hand, could not multiply in this medium unless supplied with available carbon compounds by the algae. One could, therefore, determine whether the algae were supplying the *Azotobacter* with a carbon and energy source by determining whether or not the *Azotobacter* cells multiplied under the above conditions. This test is highly sensitive and would indicate the presence of very small amounts of available carbon compounds. In other experiments, algal cultures were incubated for two or more months and filtered to remove the algal cells; the filtrates were inoculated with *Azotobacter* to determine whether these organisms could multiply in them. Finally, studies were made of the ability of *Azotobacter* to multiply in suspensions of dead algae.

The following algal cultures² were used:

- | | |
|-------------------------|-----------------------------------|
| 1. <i>Stichococcus</i> | 12. <i>Chlorococcum</i> |
| 2. <i>Chlorococcum</i> | 19. <i>Protococcus</i> -like form |
| 3. <i>Chlorococcum</i> | 20. <i>Chlorococcum</i> |
| 5. <i>Chlorococcum</i> | 22. <i>Chlorella vulgaris</i> |
| 11. <i>Chlorococcum</i> | |

For convenience, these cultures will be referred to by number. Two species of *Azotobacter*,³ *Az. vinelandii* and *Az. chroococcum*, were used. To insure the

² Obtained from Dr. C. E. Skinner, department of bacteriology, University of Minnesota, Minneapolis, Minnesota.

³ Obtained from the culture collection of the department of soil chemistry and bacteriology, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

purity of the cultures, the organisms were reisolated by repeated plating and culturing from well-isolated colonies.

The mineral salt medium used throughout this work had the following composition:

K_2HPO_4	0.5 gm.
$NaNO_3$	0.5 gm.
$MgSO_4 \cdot 7H_2O$	0.2 gm.
$CaCl_2$	0.05 gm.
$FeCl_3 \cdot 6H_2O$	trace
Distilled H_2O	1000 cc.

The numbers of algal cells were determined by direct microscopic counts using a Levy counting chamber. The *Azotobacter* cells were counted by plating on Lipman's medium (24, p. 112-113). Organic carbon was determined by the Schollenberger method in which the organic matter was oxidized by hot chromic acid and the excess acid titrated with $Fe(NH_4)_2(SO_4)_2$.

Artificial illumination was provided by a bank of five 100-watt electric bulbs approximately 2.5 feet above the cultures. No attempt was made to control quantitatively the amount of light used. As a rule, the cultures were illuminated for 8 or 9 hours in each 24-hour period.

Since similar results were obtained with both species of *Azotobacter*, only the data concerned with *Az. chroococcum* will be presented. The stock culture solution, in 100-cc. quantities, was placed in 250-cc. Erlenmeyer flasks which had been carefully cleaned with hot chromic acid, and the medium was autoclaved. Seven-day agar slant cultures of algal strains 1, 3, 5, 11, 12, 19, 20, and 22 were washed off with sterile distilled water, and 1 cc. of the resulting suspension was placed in a flask containing the sterile medium. All these flasks, as well as controls, were inoculated simultaneously with 0.2 cc. of a suspension of *Az. chroococcum*. The cultures were incubated at room temperature under light, and analyses of algal and bacterial numbers were made at stated intervals. The results are recorded in table 1.

All the algal strains multiplied extensively. This was to be expected, since all their nutritional requirements were provided. Although the rate of multiplication of the different strains varied considerably, there were a million or more cells per cubic centimeter of culture at the end of 42 days of incubation. Microscopic examinations showed that the algal cells were normal in appearance, with the exception of those of strain 12, which contained a considerable number of exceptionally large cells that were somewhat chlorotic. Toward the end of the incubation period, cultures 11, 19 and 20 were difficult to count because the cells had a pronounced tendency to clump.

Small increases in numbers of *Azotobacter* occurred in all the algal cultures. These increases amounted mostly to 200-300 thousand bacteria or less per cubic centimeter; they could hardly be considered as significant, since a similar increase took place in the control flask which did not contain any algae. The only exception was in the case of those *Azotobacter* cells which were in contact

with strain 5, when a somewhat higher count (752,000 bacteria per cubic centimeter) was obtained after 28 days of incubation. It is important to mention the fact that at no time was there any macroscopically visible sign of bacterial growth in any of the cultures. The liquid medium above the sedimented algal cells remained unclouded throughout the 42 days of incubation. Microscopic examinations made at the time of counting the algae revealed few

TABLE 1
Development of Az. chroococcum in growing algal cultures
Thousands of cells in 1 cc. of culture

ALGAL STRAIN		DAYS OF INCUBATION					GLUCOSE ADDED AT END OF 42 DAYS, 0.5 GM./FLASK
		6	13	20	28	42	
1	<i>Azotobacter</i>	138	237	277	344	4	3,800
	Algae	...	850	1,520	2,300	3,000	
3	<i>Azotobacter</i>	132	283	294	288	304	4,000
	Algae	...	290	640	600	1,950	
5	<i>Azotobacter</i>	137	360	246	752
	Algae	...	380	1,010	1,420	1,330	
11	<i>Azotobacter</i>	200	381	184	241	204	3,200
	Algae	...	160	320	1,050	*	
12	<i>Azotobacter</i>	183	274	265	250	176	2,300
	Algae	...	200	380	2,150	3,840	
19	<i>Azotobacter</i>	31	51	40	42	20	11,000
	Algae	...	1,190	2,190	3,300	*	
20	<i>Azotobacter</i>	36	66	98	85	82	3,700
	Algae	...	910	1,300	2,940	*	
22	<i>Azotobacter</i>	36	104	418	360	480	10,000
	Algae	...	1,320	2,380	4,400	7,000	
Control	<i>Azotobacter</i>	133	373	276	324	280	7,200

* Cells too badly clumped to count.

Azotobacter cells in the counting chamber, and a rough estimation of these indicated that the plate counts were good indexes of the actual number of *Azotobacter* in the cultures.

Since the algae had little or no effect on the development of *Azotobacter* in the mixed cultures, it seemed advisable to determine whether the experimental conditions were suitable for bacterial growth, if a readily available source of energy were supplied. The addition of 0.5 gm. of sterile glucose to each culture after 42 days of incubation produced a rapid and marked change in the

appearance of the cultures. In a short time, the liquid above the sedimented algae became turbid, and counts made 3 days after the addition of the glucose showed 2 to 11 millions of *Azotobacter* in each cubic centimeter of the cultures. If the incubation period had been longer, a much greater number of *Azotobacter* cells would have been found.

It must be concluded from these results that the algal strains used, under conditions favorable for their growth, supplied *Az. chroococcum* with little or no available organic compounds. The lack of an adequate supply of available energy was the factor limiting the growth of *Azotobacter*, since the addition of a small amount of glucose immediately brought about their rapid and extensive multiplication.

In order to obtain some idea as to the amounts of organic matter excreted by algae into the medium and whether this organic matter can be utilized by *Azotobacter* in the complete absence of algal cells, the following experiment was carried out.

Liter flasks containing 500 cc. of the stock medium were inoculated with algae 1, 2, 3, 5, 11, 12, 19, 20, and 22. Uninoculated flasks were kept as controls. After 51 days' incubation, the cultures were made up to the original volume with distilled water. A few cubic centimeters of each culture were withdrawn for algal counts; 100-cc. portions were used for organic carbon and Kjeldahl nitrogen determinations. The remainder of each culture was filtered through Whatman paper #2 to remove most of the algae. The algae of strains 3, 5, 11, 12, and 22 were subsequently carefully removed from the filter paper, suspended in distilled water, autoclaved, and saved for use in a later experiment. After filtration through paper, the cultures were passed through sterile Seitz pads to remove the remaining algae and to obtain sterile filtrates with as little chemical or physical change as possible. Portions of these filtrates were placed, aseptically, in sterile 125-cc. flasks. These flasks were inoculated with *Az. chroococcum* (927 cells per flask), incubated in the dark, and plated out at intervals in order to determine the changes in bacterial numbers. The remaining portions of the filtrates were used for organic carbon and pH determinations. The controls were treated in a manner identical with that used for the algal cultures. The results of this experiment are recorded in table 2.

The algal strains made extensive growth. The amounts of organic carbon in the algal filtrates were very small, being in all cases less than 1 mgm. per 100 cc. of filtrate. With the exception of strains 1 and 19, the organic carbon in the filtrates was less than 10 per cent of the total carbon synthesized by the algal cells. Apparently the algae do not liberate very much organic matter into the medium. Similar results were obtained by Roberg (22) and by Krogh, Lange, and Smith (17). The latter, working with fresh-water algae, found that the organic material synthesized by the algae was almost quantitatively stored in their cells while a fraction amounting at most to 10 per cent was dissolved in the surrounding medium.

The *Azotobacter* inoculated into the sterile algal filtrates multiplied somewhat

in most of the filtrates. The highest count obtained was 670,000 bacteria per cubic centimeter from the filtrate of strain 11.

Multiplication did not occur in the filtrates of strains 5 and 20. It appears that *Azotobacter* was able to utilize, in most cases, a small portion of the organic matter present in the filtrates.

TABLE 2
Development of Az. chroococcum in filtrates of algal cultures

ALGAL STRAIN	ALGAE*	ORGANIC CARBON IN 100 CC. OF CULTURE	ORGANIC CARBON IN 100 CC. OF FILTRATE†	PER CENT OF CARBON IN FILTRATE	NITROGEN IN 100 CC. OF CULTURE	pH OF FILTRATE	AZ. CHROOCOCCUM*		
							Days of incubation		
							2	6	15
		mgm.	mgm.		mgm.				
1	5,130	3.84	0.75	19.5	7.73	.2	265	12.6
2	1,620	7.56	0.69	9.1	7.75	<.1	30	320
3	1,590	8.64	0.57	6.6	7.85	9.3	360	Contam- inated
5	1,750	9.66	0.57	5.9	7.97	<.1	<.1	0
11	4,340	10.32	0.62	6.0	0.91	8.05	574	670
12	3,900	10.02	0.72	7.2	0.82	7.98	116	116
19	2,180	6.36	0.86	13.5	0.82	367	110	300
20	1,410‡	8.32	0.61	7.3	0.51	<.1	<.1	.002
22	4,360	7.66	0.62	8.1	0.72	7.90	103	343	67
Control			0.36	0.15	7.45	<.1	<.1	.001

* Thousands per cubic centimeter.

† Blank subtracted.

‡ Only approximate count because of clumping.

TABLE 3
Development of Az. chroococcum in suspensions of dead algae
Thousands per cubic centimeter

INCUBATION PERIOD	ALGAL STRAINS					Control
	3	5	11	12	22	
days						
2	367	293	46	89	603	<.1
6	320	600	100	188	1,360	<.1
15	469	25	Contaminated	2,010	71	.001

An attempt was finally made to determine whether *Az. chroococcum* could utilize dead algal cells as a source of energy. The autoclaved algal suspensions of strains 3, 5, 11, 12, and 22, obtained in the previous experiment were used. Two-cubic-centimeter portions of these suspensions were added to flasks containing the stock medium and autoclaved. The flasks were inoculated with *Az. chroococcum* (927 cells per flask) and incubated. The control flasks contained only the stock medium. *Azotobacter* counts were made at intervals.

The results presented in table 3 show that *Azotobacter* multiplied at the ex-

pense of the algal cells, reaching a concentration of 2,010,000 cells per cubic centimeter within 15 days, in the presence of algal strain 12, and a concentration of 1,360,000 cells per cubic centimeter within 6 days with algal strain 22. The poorly developed saprophytic character of *Azotobacter*, insofar as the utilization of the organic matter of dead algae is concerned, was strikingly indicated in this experiment. Normally, the flasks containing the autoclaved algae showed no macroscopically visible signs of *Azotobacter* growth, since the liquid above the sedimented algae remained clear and the algal cells retained their initial appearance and green coloring. Occasionally, however, a flask would suddenly show marked turbidity, and the mass of algal cells would appear to be undergoing decomposition. Microscopic examinations always revealed that the extensive growth was due to a bacterial contaminant. Undoubtedly, *Azotobacter* can obtain some energy from the organic matter of dead algae, although the rate of utilization is slow.

ABILITY OF CERTAIN ALGAE TO FIX NITROGEN

During the last decade, it has definitely been proved that some species of blue-green algae can fix atmospheric nitrogen. Drewes (8) isolated *Nostoc punctiforme* and *Anabaena variabilis* in pure culture and showed that these algae fixed 2 to 3 mgm. of nitrogen when inoculated into 250 cc. of nitrogen-free medium and incubated for 50 days. Allison and co-workers (1) have shown that *Nostoc muscorum*, under certain conditions, was able to fix as much as 18 mgm. of nitrogen per 100 cc. of medium in 85 days. Of great importance was the fact that this organism was found to form normal chlorophyll in the dark and to fix 10 to 12 mgm. of nitrogen per gram of glucose consumed. This compared favorably with the activity of *Azotobacter* and indicated that *Nostoc muscorum* may be of considerable importance in enriching soils with nitrogen. Recently, De (7) reported the isolation of pure cultures of nitrogen-fixing blue-green algae, *Anabaena variabilis*, *A. gelatinosa*, and *A. naviculoides* from Indian soils.

A culture of *Nostoc muscorum*⁴ was checked for purity and for the nitrogen-fixing ability of this organism. The claims of Allison and co-workers were fully confirmed. Cultural and direct microscopic tests for contaminating microorganisms were uniformly negative. Definite fixation occurred in the light, and in the dark, in the presence of glucose.

In order to evaluate the importance of nitrogen-fixing algae in the nitrogen economy of soils, it was essential to determine how frequently these organisms occur in soils. Approximately 80 samples of soil, including garden, field, pasture, greenhouse, and orchard soils as well as soils from experimental plots at the N. J. Agricultural Experiment Station, were tested for the presence of nitrogen-fixing algae. Two-gram portions of each soil were inoculated into flasks containing 50 cc. of algal medium to which nitrogen had not been added.

⁴ Obtained from Dr. F. E. Allison.

The cultures were incubated under electric lights and examined frequently. Appreciable growth of blue-green algae was noted in 35 cases after incubation periods of three or more weeks. As a rule, the appearance of the blue-green algae was preceded by the development of unicellular grass-green forms. Whenever blue-green algae appeared in the flask, a small portion of the growth was taken from the culture, washed in three changes of distilled water to remove as much contamination as possible, and reinoculated into a fresh flask of nitrogen-free medium. In this way, the complicating effect of the nitrogen added to the first culture by the soil was eliminated. On subculture, only 2 of the 35 cultures were able to grow. The remaining 33 cultures developed only when fixed nitrogen (KNO_3) was added to the medium. The addition of molybdenum and vandadium to nitrogen-free media failed to induce their growth. These were, therefore, considered as being unable to utilize atmospheric nitrogen.

One of the two cultures subcultured in nitrogen-free media, No. C-5, was obtained from a cylinder experiment in which the soil had been fertilized with

TABLE 4
Fixation of nitrogen by cultures of blue-green algae isolated from soil

CULTURE	TOTAL NITROGEN IN 100 CC. OF CULTURE	NITROGEN FIXED IN 100 CC. OF CULTURE
	mgm.	mgm.
Uninoculated control	0.2	...
C-5	4.5	4.3
6236	2.7	2.5

$(\text{NH}_4)_2\text{SO}_4$, and the other, No. 6236, was isolated from a greenhouse soil. Microscopic examination revealed that both cultures were of the *Nostoc* type.

In order to test the ability of the cultures to fix atmospheric nitrogen, the two cultures of blue-green algae were again subcultured in nitrogen-free media and, after 42 days of incubation, analyzed for total nitrogen. Uninoculated media served as controls. At the time of making the nitrogen analyses, the cultures were tested for the presence of *Azotobacter* by streaking some of the material on the surface of agar plates of Lipman's medium. *Azotobacter* cells were not found in any of the cultures. Both cultures fixed appreciable amounts of nitrogen, as shown in table 4. On the basis of the following facts, it seems definite that the blue-green algae in these two cultures were responsible for the nitrogen fixed: (a) the algae were able to make extensive growth in media free of added nitrogen; (b) chemical analyses proved that appreciable quantities of nitrogen were fixed by these cultures; (c) *Azotobacter* could not be demonstrated; (d) the blue-green algae present were of the *Nostoc* type, species of which have been shown to be able to fix atmospheric nitrogen. It appears that although blue-green algae occur in many soils, only a small number of these possess the ability to utilize free nitrogen.

SUMMARY AND CONCLUSIONS

In the presence of actively growing cultures of nine strains of grass-green algae, *Az. chroococcum* failed to make appreciable growth under conditions where the sole limiting factor was a supply of available carbon and energy. One must conclude that the algae supplied *Azotobacter* with little or no available organic matter.

Algae liberated small amounts of organic compounds into the surrounding medium. In most cases, this amounted to less than 10 per cent of the total carbon assimilated by the algae. This accounts partly for the lack of marked development of *Azotobacter* in algal cultures; however, the most important probable factor in connection with the limited growth of the bacterium is the unavailability of even this part of the organic matter.

Some multiplication of *Azotobacter* occurred when this organism was inoculated into filtrates of algal cultures. This, however, was slow and limited in extent. Somewhat greater multiplication took place in the autoclaved algal suspensions, but this too was slow when compared with the rapid development of other bacteria that occasionally found their way, as contaminants, into the suspensions of algae.

Qualitatively, the conception of a beneficial symbiosis between algae and *Azotobacter* may be true, since the two organisms do not appear to be antagonistic and since small increases in numbers of *Azotobacter* occurred in some cases; however, because of the extreme sensitivity of the biological method used, these increases must be interpreted with caution. Quantitatively, the degree of symbiosis appears to be very slight.

The indirect role of the majority of soil algae in the fixation of atmospheric nitrogen either is nonexistent or functions to only a very small extent, for despite favorable environmental conditions and the presence of large numbers of algae, *Azotobacter* could not make appreciable growth with the amount of energy supplied by the algae. These optimum conditions rarely occur in nature where *Azotobacter* has to compete with numerous microbial forms such as fungi, actinomycetes, and other bacteria for the available organic matter.

Blue-green algae must be considered as direct participants in the fixation of nitrogen in the soil. Their practical importance, however, still remains to be determined.

REFERENCES

- (1) ALLISON, F. E., HOOVER, S. R., AND MORRIS, H. J. 1937 Physiological studies with the nitrogen-fixing alga, *Nostoc muscorum*. *Bot. Gaz.* 98: 433-463.
- (2) ALLISON, F. E., AND MORRIS, H. J. 1930 Nitrogen fixation by blue-green algae. *Science* 71: 221-223.
- (3) ALLISON, F. E., AND MORRIS, H. J. 1932 Nitrogen fixation by soil algae. *Proc. Second Internatl. Cong. Soil Sci. III Comm.* 3: 24-28.
- (4) BOUILHAC, R. 1896 Sur la fixation de l'azote atmosphérique par l'association des algues et des bactéries. *Compt. Rend. Acad. Sci. [Paris]* 123: 828-830.

- (5) BOUILHAC, R., AND GIUSTINIANI 1903 Sur une culture de sarrasin en présence d'un mélange d'algues et des bactéries. *Compt. Rend. Acad. Sci. [Paris]* 137: 1274-1276.
- (6) BOUILHAC, R., AND GIUSTINIANI 1904 Sur des cultures de diverses plantes supérieures en présence d'un mélange d'algues et des bactéries. *Compt. Rend. Acad. Sci. [Paris]* 138: 293-296.
- (7) DE, P. K. 1939 The role of blue-green algae in nitrogen fixation in rice fields. *Proc. Roy. Soc. [London]* (B) 127: 121-139.
- (8) DREWES, K. 1928 Über die Assimilation des Luftstickstoff durch Blaualgen. *Centbl. Bakt. (II)* 76: 88-101.
- (9) EMERSON, P. 1918 Soil inoculation with *Azotobacter*. *Iowa Agr. Exp. Sta. Res. Bul.* 45: 27-64.
- (10) FISCHER, HERM. 1916 Über qualitative und quantitative Leistungen stickstoffsammelnder Bakterien im Wasser und im Boden unter Wasserbedeckung. *Centbl. Bakt. (II)* 46: 304-320.
- (11) FISCHER, HUGO 1904 Über Symbiose von *Azotobacter* mit *Oscillarien*. *Centbl. Bakt. (II)* 12: 267-268.
- (12) FRANK, B. 1889 Über den experimentellen Nachweis der Assimilation freien Stickstoffs durch erdbewohnende Algen. *Ber. Deut. Bot. Gesell.* 7: 34-42.
- (13) HEINZE, B. 1906 Einige Beiträge zur mikrobiologischen Bodenkunde. *Centbl. Bakt. (II)* 16: 640-653, 703-711.
- (14) HEINZE, B. 1906 Über die Stickstoffassimilation durch niedere Organismen. *Landw. Jahrb.* 35: 889-910.
- (15) JONES, J. 1930 An investigation into the bacterial associations of some Cyanophyceae with especial reference to their nitrogen supply. *Ann. Bot.* 44: 721-740.
- (16) KOSSOWITSCH, P. 1894 Untersuchungen über die Frage, ob die Algen freien Stickstoff fixieren. *Bot. Ztg.* 52: 97-116.
- (17) KROGH, A., LANGE, E., AND SMITH, W. 1930 On the organic matter given off by algae. *Biochem. Jour.* 24(2): 1666-1671.
- (18) KRÜGER, W., AND SCHNEIDEWIND, W. 1900 Sind neidere chlorophyllgrüne Algen imstande, den freien Stickstoff den Atmosphaere zu assimilieren und den Boden an Stickstoff zu bereichern. *Landw. Jahrb.* 29: 771-804.
- (19) LIPMAN, C. B., AND TEAKLE, L. J. H. 1925 Symbiosis between *Chlorella* sp. and *Azotobacter chroococcum* and nitrogen fixation. *Jour. Gen. Physiol.* 7: 509-511.
- (20) NAKANO, H. 1917 Untersuchungen über die Entwicklungs- und Ernährungsphysiologie einiger Chlorophyceen. *Jour. Col. Sci., Imp. Univ. Tokyo* 40: 1-240.
- (21) REINKE, J. 1903 Symbiose von *Volvox* und *Azotobacter*. *Ber. Deut. Bot. Gesell.* 21: 481-483.
- (22) ROBERG, M. 1930 Ein Beitrag zur Stoffwechselphysiologie der Grünalgen. *Jahr. Wiss. Bot.* 72: 369-384.
- (23) SCHLOESING, TH. FILS, AND LAURENT, EM. 1891 Sur la fixation de l'azote libre par les plantes. *Compt. Rend. Acad. Sci. [Paris]* 113: 776-779.
- (24) WAKSMAN, S. A. 1927 Principles of Soil Microbiology. Williams and Wilkins Co., Baltimore, Md.

PRIMARY MINERALS OF THE SILT FRACTION AS CONTRIBUTORS TO THE EXCHANGEABLE-BASE LEVEL OF ACID SOILS¹

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The value to plants of a high level of exchangeable bases in the soil has become evident. Early recognition came from studies of the growth and nitrogen fixation of soybeans in relation to the amounts and degrees of saturation of nutrient cations in this form. In many of our permanently acid soils, this exchangeable-base level has dropped to a dangerously low point. In others it is rapidly moved there. It thus becomes important that all possible sources of mineral bases be considered and investigated.

Studies by Albrecht, Graham, and Ferguson (1), suggested that the breakdown of the inorganic colloidal clay fraction of Putnam silt loam would be insignificant in providing the mineral base requirement of legume plants growing on the clay. Since the sand fraction of soil is composed primarily of quartz, this would leave the primary minerals of the silt fraction as the only source of mineral bases. Jenny, Cowan (4), and others have shown that when a base leaves the ionic atmosphere of the colloidal fraction the hydrogen ion takes its place. The question now arises as to whether a base can transfer from the crystal lattice of the primary mineral in the silt fraction to the ionic atmosphere of the colloidal fraction. It was under the assumption that this takes place that the following investigation was undertaken.

PLAN OF STUDY

Crystal pure samples of quartz, augite, microcline, biotite, anorthite, and hornblende were each ground in a ball mill until they had reached a fine state of subdivision. Each sample was then placed in a liter cylinder, distilled water was added, and the fraction ranging in size from 0.05 to 0.005 mm. equivalent diameter was separated by means of the beaker method of mechanical analysis. The silt fraction so obtained was then placed in a Gooch crucible and 1 liter of 0.001 *N* HCl was allowed to leach slowly through it. The sample was then washed free of chlorides. The pH of the aqueous solution of the leached mineral was used as the index to insure that all the basic cations

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released by grinding had been removed. The mineral sample was then oven dried and reserved for later treatment.

Colloidal clay was extracted from the heavy subsoil layer of Putnam silt loam and electrodyalized according to the method of Bradfield (2). The clay suspension thus prepared, containing 3.99 per cent of dry material, had a pH of 3.3. A conductometric determination of the exchange capacity according to the carbonation method of Bradfield and Allison (3) showed this to be 68 m.e. per 100 gm. of clay.

The mixtures of acid clay and finely ground minerals were prepared by taking units of the hydrogen clay suspension to give 5 gm. of clay and adding to each the 10 gm. of one of the silt samples of the primary minerals previously prepared. The mixtures were shaken thoroughly and allowed to stand for 31 days, when the pH of each mixture was determined. This was repeated after 70 days and again at the end of 107 days.³ After this time interval the mixtures were transferred to large centrifuge tubes. Neutral ammonium acetate was added, and the mixtures were centrifuged. The clear supernatant solution was siphoned off. The ammonium acetate washing was repeated four times to insure the removal of all the exchangeable cations. The supernatant liquid was analyzed by standard quantitative procedures for its sesquioxides, calcium, and soluble material other than these. The small amounts of silicon made soluble were removed by dehydration and filtration.

Samples of three of the primary minerals—augite, hornblende, and anorthite—in quantities used in the foregoing treatments were mixed with the same amount of ordinary distilled water as that contained in the suspension. After these aqueous mineral mixtures had stood for 70 days they were analyzed by standard procedures for iron and calcium. This part of the experiment was arranged to test the mineral breakdown in the presence of water only.

EXPERIMENTAL RESULTS

The pH of the mineral colloidal-clay mixtures increased decidedly in some instances and insignificantly in others. This is evident in figure 1. The pH of the anorthite-clay mixture showed the greatest change, from 3.3 to 5.70 after 107 days.

The analyses of the supernatant liquids obtained by the addition of normal ammonium acetate showed that no sample contained more than a trace of sesquioxides. The results of the calcium analysis, presented in table 1, show that calcium was present in the extract from every sample. The exchangeable calcium obtained from the anorthite-clay mixture represented 3.4 per cent of the total calcium present in the 10 gm. of crystal sample used.

Four of the samples contained only traces of soluble materials other than calcium, but the anorthite-clay and the hornblende-clay mixtures contained significant amounts. The data are assembled in table 1.

³ The pH determinations at the end of the 70-day interval were made with a glass electrode; all others were made with quinhydrone.

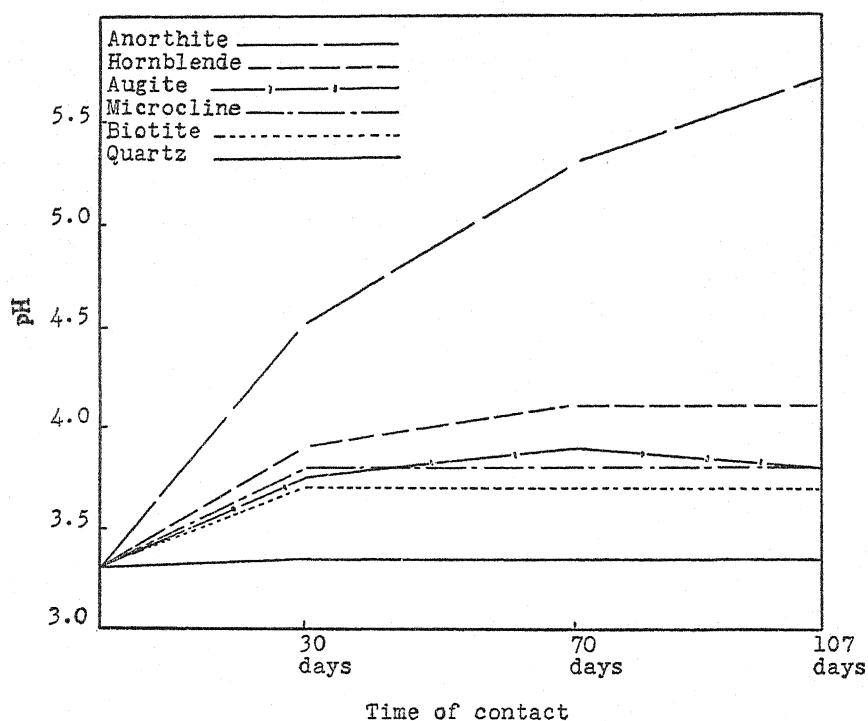


FIG. 1. CHANGES WITH TIME IN THE pH OF MINERAL COLLOID-CLAY MIXTURES

TABLE 1

Exchangeable calcium and other soluble materials in mixtures of clay with minerals

MIXTURE	CALCIUM	SOLUBLE MATERIALS OTHER THAN CALCIUM
	<i>mgm.</i>	<i>mgm.</i>
Quartz.....	1.3	Trace
Augite.....	7.1	Trace
Microcline.....	5.1	Trace
Biotite.....	2.8	Trace
Hornblende.....	10.3	14.5
Anorthite.....	48.1	7.5

TABLE 2

Iron and calcium found in the filtrate of aqueous mineral mixtures after 70 days

MIXTURE	IRON	CALCIUM
	<i>mgm.</i>	<i>mgm.</i>
Augite.....	1.98	0.0
Hornblende.....	0.0	3.0
Anorthite.....	0.0	0.5

Data on the mineral breakdown in water, as measured by an analysis of the filtrate from the aqueous mineral mixture, are shown in table 2.

DISCUSSION

The results obtained in this study show a definite transposition or metathesis of calcium from the crystal lattice of the anorthite, hornblende, and augite to the ionic atmosphere of the colloidal clay. The potash feldspar, microcline, proved to be almost invulnerable to the action of the hydrogen ions. The biotite mica was even more resistant than the microcline. Field evidence points toward this order of weathering but gives no indication that it can go on as rapidly as indicated in this experiment. Here the hydrogen of the acid clay released 3.4 per cent of the total calcium in anorthite in 107 days as compared to 0.03 per cent of the total calcium by simple hydrolysis in 70 days.

The silt fraction of the very acid Putnam silt loam has a mineral composition of approximately 20 per cent, other than quartz, which is composed of muscovite, albite, and potash feldspars. A comparison might be made with the silt fraction of the neutral Marshall silt loam, which according to Robinson (5) has a mineral composition of approximately 34 per cent, other than quartz, which consists of potash feldspar, muscovite, biotite, magnetite, epidote, albite, labradorite, oligoclase, tourmaline, rutile, glaucophane, hornblende, and augite. The Marshall soil, in contrast with Putnam silt loam, contains the calcium feldspars; namely, labradorite, oligoclase, hornblende, and augite, which can furnish bases to replenish the supply of the colloidal fraction. It is not surprising then that the pH of the Marshall soil is near 6.9, whereas the pH of the Putnam is 5.0, and that this latter soil has a "lime requirement" of 6000 pounds per acre.

Robinson (5) reported that Volusia silt loam (a well-known acid soil) has a silt fraction of minerals which are 38 per cent other than quartz. On a basis of other-than-quartz minerals, we might expect this soil to have a high exchangeable-base level. The minerals which made up the other-than-quartz fraction, however, were mainly potash feldspar, with small amounts of muscovite, rutile, epidote, biotite, tourmaline, augite, and hornblende. The calcium-bearing feldspars were not found. These facts coincide with the observation on the stability of microcline in the presence of hydrogen clay. Consequently, we may have acid soils of which the silt fraction contains large amounts of other-than-quartz minerals. It also appears that any soil which is void of calcium feldspars would necessarily be unable to maintain a high exchangeable-calcium level. To maintain the exchangeable-calcium level on soils of this latter type, calcium must be added in the form of limestone or fertilizers.

The data show that the hydrogen colloid is a very active force in the weathering of the calcium-bearing minerals. In these trials it was so active that 3.4 per cent of the total calcium present in the anorthite was moved out of

the crystal into the exchange envelope. This represents an activity approximately one hundred times as great as that of hydrolysis on anorthite by ordinary distilled water.

The method used in this experiment points to many possibilities for future study. Soil or mineral weathering, mechanisms of exchange of cations, conditions and time intervals significant in soil fertility, and numerous other activities could be studied by this method and from this point of view. Further studies can doubtless give some accurate measurements of changes in mineral structure and weathering rates.

SUMMARY AND CONCLUSIONS

This study of the influence of hydrogen clay on the metathesis of the bases from the structure of primary minerals of the silt size revealed the following:

In the comparatively short time interval of 107 days, calcium can be transposed from the crystal of anorthite to the exchange atmosphere of hydrogen colloidal clay in such quantities as to change the pH of soil clay from 3.30 to 5.70.

The hydrogen of the colloid can be effective in bringing about calcium removal also from the crystals of hornblende and augite.

Biotite and microcline are little affected by the action of the hydrogen sorbed in the exchange atmosphere of colloid clay.

The action of sorbed hydrogen ions of the clay brought about a weathering effect on the mineral crystal significant enough to remove 3.4 per cent of the total calcium held in the anorthite mineral.

The hydrogen clay thus becomes a very active agent in the weathering processes when it causes approximately 100 times as much calcium to be removed from anorthite as was removed by the hydrolytic action of water.

REFERENCES

- (1) ALBRECHT, W. A., GRAHAM, E. R., AND FERGUSON, C. E. 1939 Plant growth and the breakdown of inorganic soils colloids. *Soil Sci.* 47: 455-458.
- (2) BRADFIELD, R. 1923 The chemical nature of colloidal clay. Missouri Agr. Exp. Sta. Res. Bul. 60.
- (3) BRADFIELD, R., AND ALLISON, W. H. 1933 Criteria of base saturation of soils. *Trans. Second Comn. and Alkali Subcomn. Internatl. Soc. Soil Sci.* A: 63-79.
- (4) JENNY, H., AND COWAN, E. W. 1933 The utilization of adsorbed ions by plants. *Science* 77: 394-396.
- (5) ROBINSON, W. O. 1914 The inorganic composition of some important American soils. U. S. Dept. Agr. Bur. Soils Bul. 122.

SURVIVAL OF MICROORGANISMS INTRODUCED INTO SOIL¹

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When an organism is introduced artificially into soil it must adjust itself to its new environment if it is to survive and multiply. Successful inoculation is dependent primarily on the persistence and development of the inoculant in the soil; hence it is essential that the addition of an organism to soil be preceded by careful consideration of the conditions which will make this substrate suitable for it. Such factors as food supply (organic and inorganic), temperature, oxygen tension, moisture content, reaction (pH), and associative and antagonistic relationships (25) merit close attention.

Rhizobia have been used most extensively for soil inoculation (7). It was found, for example, that *Rh. leguminosarum* and *Rh. meliloti* persisted in much greater numbers in fertilized than in untreated soil (19). As *Rh. meliloti* prefers a neutral to alkaline reaction, liming an acid soil is an important practice where this bacterium is to be introduced. Many other examples might be cited which supply ample proof of the importance of soil amelioration in establishing Rhizobia in soil (7).

Azotobacter cultures have been widely used as soil inoculants (9, 24), but the results have not been particularly favorable, possibly because of the difficulty of establishing the organisms in the soil. Correction of unfavorable conditions, whether by liming or addition of carbohydrates or both, enabled the organisms to persist and develop in soils from which, theretofore, they had disappeared rapidly after being introduced (14).

An increasingly important phase of soil inoculation is that which relates to the use of organisms antagonistic to soil-borne pathogens. Again it is essential to establish the control organism in its new habitat before it can be expected to exert its antagonistic effects. There is some evidence of biological control of certain plant parasites by the introduction of antibiotic forms into the soil (2, 11, 12, 16, 20, 27, 28); this protection is not universal, however, partly because of the difficulty of establishing the antagonistic form in soil (5, 6).

In the present investigation, certain typical soil fungi, bacteria, and actinomycetes were inoculated into soils, and studies were made of their survival over a period of time.

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EXPERIMENTAL

Survival of organisms inoculated into soils

Palouse silt loam and soils taken from the New Jersey Agricultural Experiment Station plots 5A, 5B, 7A, and 7B, which had received various field treatments, were used (14). The soils were sieved, placed in 300-gm. portions in glass jars, brought to 50 per cent of the moisture-holding capacity, and inoculated with suspensions of the organisms. Only those species of organisms which could be easily recognized and readily distinguished from the other forms developing on the plates were employed. In the case of bacteria, young agar cultures were employed; spore suspensions were used for fungi; and mixtures of spores and mycelium, for actinomycetes. Samples of treated soils were removed for plating 24 hours after inoculation and at periodic intervals thereafter. Nutrient and sodium-albuminate agars were used for bacteria and actinomycetes, and peptone-acid agar for fungi (8). All the jars were weighed, covered, and incubated at 28°C.; moisture was maintained at the optimum by weekly additions of water.

The method used for detecting the organisms introduced had certain limitations. It is difficult, for instance, to distinguish certain organisms on too crowded plates, as is the case with low dilutions, 1:100 to 1:1000 for fungi and 1:1000 to 1:10,000 for bacteria and actinomycetes. Consequently, when a statement is made that an organism, originally present in large numbers as a result of inoculation, has disappeared, the implication is that fungi were absent from plates with soil dilutions of 1:100 to 1:1000, and bacteria and actinomycetes from plates in dilutions of 1:1000 to 1:10,000. Such absence is indicated by a zero in the tabular data. Obviously the organisms in question may have been present in smaller numbers, but the method employed in this study was not sufficiently exact to detect them.

The survival of a group of organisms introduced into the various soils is presented in tables 1 to 3. The microbial flora of the uninoculated soils reflected to a certain extent the characteristics and previous history of the soils used. Palouse soil, the highest in nitrogen and organic matter, supported larger numbers of bacteria and actinomycetes than the other soils, with the exception of 5B which had a bacterial count as high as that of the Palouse. Because of its high acidity, soil 7A had the smallest numbers of bacteria and actinomycetes. Soil 7B with a higher pH value gave counts of microorganisms approaching those of soils 5A and 5B. Fungus counts were not widely divergent in the soils, although it might have been expected that the soils with lower pH would have a higher fungus flora (22, 23). Possibly the air-drying of the soils before plating was responsible for some of these unexpected effects.

The most significant and consistent results obtained with all the soils was the complete disappearance, within the experimental period, of three organisms, *F. culmorum*, *Act. cellulosa*, and *Ps. fluorescens*. To be sure, the other organisms decreased in numbers as well, but none of those studied in all the

soils disappeared within the time of the experiment. In the Palouse soil, *Helminthosporium* disappeared; because of the difficulty encountered in dis-

TABLE 1
Survival of microorganisms inoculated into Palouse soil
Numbers per gram oven-dry soil, $\times 10^{-5}$

ORGANISM	DAYS OF INCUBATION								
	0	5	13	22	32	46	83	167	230
<i>Cunninghamella</i> sp.....	0.8	0.5	0.6	0.8	0.3	0.7	0.6	0.8	0.2
<i>Aspergillus</i> sp.....	3.6	3.6	3.4	2.6	3.5	3.9	3.1	2.4	1.5
<i>Penicillium</i> sp.....	23.1	17.1	17.2	15.9	15.6	18.6	12.9	5.8	2.9
<i>Trichoderma</i> sp.....	4.5	11.5	9.9	5.5	8.3	8.8	8.9	3.1	3.6
<i>Actinomyces cellulosae</i>	18.6	10.4	13.3	11.1	4.1	3.2	0.5	0, 4.1*	0.0
<i>Bacillus cereus</i>	39.7	55.5	40.9	23.8	46.1	42.1	34.3	45.5	22.6
<i>B. megatherium</i>	9.7	20.9	41.9	10.5	16.9	25.5	36.4	14.9	21.2
<i>Pseudomonas fluorescens</i>	175.0	144.0	67.8	26.3	13.1	15.2	0, 71.9*	0, 171*	0.0
<i>Helminthosporium sativum</i> †.....	320.0	161.0	625.0	540.0	792.0	387.0	309.0	0.0	0.0
<i>Fusarium culmorum</i>	3.7	3.3	2.3	2.4	0.8	0.7	0.1	0.0	0.0
Fungi†.....	1.4	1.9	0.5	1.0	0.6	0.5	0.9	0.3	0.4
Actinomycetes†.....	30.2	52.4	29.7	40.4	43.1	31.1	28.7	54.2	34.9
Bacteria†.....	175.0	145.0	138.0	48.7	45.9	52.2	47.6	57.1	82.4

* Reinoculated.

† *H. sativum* per gram oven-dry soil.

‡ Uninoculated soil.

TABLE 2
Survival of microorganisms inoculated into unmanured, limed, and unlimed Sassafras soils
Numbers per gram oven-dry soil, $\times 10^{-5}$

SOIL	7A					7B				
	0	20	45	75	100	0	20	45	75	100
Incubation.....days										
<i>Cunninghamella</i> sp.....	0.2	0.8	0.2	0.3	0.3	0.2	0.1	0.1	0.1	0.1
<i>Aspergillus</i> sp.....	2.1	12.6	1.2	1.2	2.0	5.4	8.3	26.9	10.1	9.6
<i>Penicillium</i> sp.....	23.5	6.0	5.2	1.9	1.9	23.4	20.0	13.9	10.6	11.1
<i>F. culmorum</i>	0.2	0.1	0.0	0.0	0.0	2.8	0.1	0.0	0.0	0.0
<i>Act. cellulosae</i>	4.6	1.5	0.3	0.0	0.0	13.2	0.8	0.4	0.0	0.0
<i>B. cereus</i>	13.6	4.4	6.8	10.5	9.1	36.0	43.2	25.7	20.8	22.9
<i>B. megatherium</i>	24.1	16.0	15.8	21.8	17.3	31.4	65.3	33.7	35.6	31.5
<i>B. mycoides</i>	72.0	20.3	7.7	14.3	11.1	22.6	64.8	23.9	29.3	26.4
<i>Ps. fluorescens</i>	33.1	0.0	148.0*	0.0	0.0	477.0	3.8	0.0	0.0	0.0
Fungi†.....	0.9	1.0	0.9	0.8	0.9	0.3	1.0	0.6	0.7	0.7
Actinomycetes†.....	2.3	0.9	1.3	1.4	1.2	14.9	15.0	18.0	17.3	16.1
Bacteria†.....	9.5	4.2	3.0	2.9	3.1	30.6	38.0	26.1	27.9	26.6

* Reinoculated.

† Uninoculated soil.

tinguishing this organism on fungus plates, it was not used further. It was also difficult to make accurate counts of *Trichoderma* because of its rapid

growth and spreading habit; this organism, too, was therefore eliminated from subsequent studies.

The fungi used in this investigation are among the most common forms occurring in soils (1, 13, 17, 18, 22). *Cunninghamella* is perhaps less frequently encountered than the others, although it has been isolated from soil by various investigators (3, 17, 18, 21). *Helminthosporium* is rather widely distributed, especially when it is actively parasitic; it also has been frequently isolated from soil (3, 4, 22). It is reasonable to expect that the introduced organisms would encounter competition from the active native microflora, in addition to the adverse physical and chemical conditions of the soil itself. The integrated effect of these factors led to a rather definite decrease of numbers of some organisms and to the elimination of others, whereas in

TABLE 3
Survival of microorganisms inoculated into manured and manured and limed Sassafras soils
Numbers per gram oven-dry soil, $\times 10^{-5}$

Soil	5A					5B				
	0	20	45	75	100	0	20	45	75	100
Incubation.....days										
<i>Cunninghamella</i> sp.....	0.5	0.8	0.4	0.4	0.4	0.7	0.2	0.1	0.2	0.1
<i>Aspergillus</i> sp.....	8.5	5.1	4.0	4.9	4.0	7.5	5.1	2.1	2.5	1.9
<i>Penicillium</i> sp.....	24.7	12.9	7.7	9.6	7.1	33.9	4.4	2.7	2.5	2.2
<i>F. culmorum</i>	0.4	1.1	0.9	0.1	0.0	2.0	0.1	0.1	0.02	0.0
<i>Act. cellulosa</i> e.....	8.4	2.2	0.1	0.0	0.0	7.6	1.4	0.04	0.0	0.0
<i>B. cereus</i>	23.2	31.7	57.4	51.5	49.3	86.9	27.1	8.6	10.5	12.3
<i>B. megatherium</i>	23.4	70.5	38.2	40.6	35.3	32.1	45.0	16.2	15.3	12.4
<i>B. mycoides</i>	64.3	71.1	125.4	101.7	98.6	55.3	54.6	36.7	29.6	25.3
<i>Ps. fluorescens</i>	142.8	0.0	0.0	0.0	0.0	175.0	6.7	1.1	0.0	0.0
Fungi*.....	1.3	1.0	1.0	1.1	1.5	0.9	1.1	0.8	0.9	0.8
Actinomycetes*.....	14.4	20.8	30.6	25.1	20.7	24.2	18.2	22.6	21.7	19.9
Bacteria*.....	38.0	46.7	39.4	36.1	35.8	48.4	46.6	52.8	50.6	45.3

* Uninoculated soil.

pure culture and in sterile soil there was positive development in all cases, as shown elsewhere (15). It is interesting to note that the *Fusarium* disappeared much more rapidly from soils 7A and 7B than from the other soils, which contained more organic matter and nitrogen.

*Actinomyces cellulosa*e decreased comparatively slowly in the Palouse soil, which appeared to be more favorable for actinomycetes than the other soils; in the latter soils, this organism disappeared at about the same time.

The numbers of spore-forming bacteria did not decrease so rapidly as did the other organisms; in fact, in some cases there seems to have been an increase. Because of their ability to produce spores, these organisms are more resistant than are other bacteria. It is quite possible that they persisted in the soil chiefly as spores (24). It was impossible, however, to distinguish between the vegetative cells and the spores in the soils by the usual plate method.

The sharpest decrease in the numbers of these organisms occurred with *B. cereus* in soil 5B and with *B. mycoides* in soil 7A.

Pseudomonas fluorescens is commonly believed to be widely distributed in soils (24). Its rapid disappearance from soils 7A and 5A suggests that the reaction could be one of the limiting factors; its longer persistence in soil 5B and especially in the Palouse soil may be evidence of the favorable effect of organic matter.

Survival of microorganisms in soils receiving different treatments

It was reported previously (14) that certain soil treatments provided conditions favorable for the survival and multiplication of *Azotobacter* in soils. The same procedure was followed in an attempt to establish *Ps. fluorescens*, *F. culmorum*, and *Act. cellulosa* in the soils from which they were eliminated. The soils were treated with different materials, at the rate of 1 per cent, and designated as follows:

PS. FLUORESCENS		F. CULMORUM		ACT. CELLULOSAE	
Designation	Treatment	Designation	Treatment	Designation	Treatment
B1	Control	F1	Control	A1	Control
B2	CaCO ₃	F2	CaCO ₃	A2	CaCO ₃
B3	Alfalfa	F3	Alfalfa	A3	Alfalfa
B4	Straw	F4	Straw	A4	Straw
B5	Manure	F5	Manure	A5	Manure
B6	Dried blood	F6	Alfalfa + CaCO ₃	A6	Alfalfa + CaCO ₃
B7	Alfalfa + CaCO ₃	F7	Straw + CaCO ₃	A7	Straw + CaCO ₃
B8	Straw + CaCO ₃	F8	Manure + CaCO ₃	A8	Manure + CaCO ₃
B9	Manure + CaCO ₃				
B10	Dried blood + CaCO ₃				

Pseudomonas fluorescens decreased considerably in numbers in the Palouse soil except where dried blood had been added (table 4). The same was true in the later incubation periods (not included in the table). Next came the soil treated with alfalfa. The total numbers of soil microorganisms were also highest in the soils receiving dried blood, as shown in tables 5 and 6. There was evidently little correlation with the pH values (table 7).

In soil 7A *Ps. fluorescens* persisted wherever lime was added; addition of dried blood had a similar effect, due perhaps also to an increase in pH value. At the end of 12 days, there was a pronounced increase in number of cells of this bacterium in soil 7A treated with straw and dried blood together with CaCO₃. In soil 7B *Ps. fluorescens* disappeared after 45 days, but it persisted where calcium was added, especially in combination with organic materials. The organism persisted in soil 5A receiving lime and especially in the soil treated with dried blood.

The addition of CaCO_3 alone to soil 5B did not make conditions for the survival of *Ps. fluorescens* any more favorable than those in the control soil. In combination with dried blood and to a lesser extent with alfalfa it had a beneficial effect. The soil treated with lime and alfalfa supported a bacterial flora which was, next to the soils with dried blood, the most abundant numerically (table 6).

TABLE 4
Survival of Ps. fluorescens in soils receiving different treatments
Numbers per gram oven-dry soil, $\times 10^{-6}$

SOILS	PALOUSE			7A			7B			5A			5B		
	0	12	40	0	12	40	0	12	40	0	12	40	0	12	40
B1	18	1.3	0.1	120	0.05	0.00	98	9	0.07	80	4.5	0.0	23	2.1	0.03
B2	11	1.4	0.2	134	22.0	3.0	49	3	0.12	79	5.7	0.3	52	0.4	0.05
B3	18	8.4	0.6	144	3.0	0.1	72	29	0.13	87	0.8	0.0	34	4.7	0.11
B4	18	0.9	0.09	174	2.0	0.07	99	6	0.11	88	3.7	0.0	39	1.0	0.03
B5	16	0.6	0.05	151	1.0	0.05	81	3	0.07	93	7.9	0.0	32	1.8	0.18
B6	10	10.6	2.20	100	51.0	38.0	95	564	12.0	108	15.4	1.3	35	2.1	2.50
B7	17	4.4	1.2	110	67.0	1.0	68	158	2.5	99	88.4	0.9	43	4.4	0.51
B8	16	0.7	0.07	158	1611.0	2.0	87	5	0.1	122	14.5	0.3	59	1.7	0.01
B9	16	2.8	0.09	131	6.0	3.0	84	9	0.13	91	10.2	0.7	31	2.8	0.02
B10	14	15.0	3.0	138	604.0	35.0	90	242	18.0	115	90.0	18.0	48	2.9	2.30

TABLE 5
Influence of various treatments on the numbers of fungi in different soils
Numbers per gram oven-dry soil, $\times 10^{-4}$

SOILS	PALOUSE			7A			7B			5A			5B		
	0	12	40	0	12	40	0	12	40	0	12	40	0	12	40
F1	6	22	5	5	46	11	1.0	4.2	0.8	6.8	5.5	2.9	2.8	6.2	4.1
F2	5	13	7	4	112	12	2.0	4.4	8.9	5.8	7.3	1.8	3.4	2.0	3.3
F3	7	41	6	4	191	41	0.9	18.0	9.6	5.3	137.0	6.0	5.5	31.0	3.0
F4	4	22	26	6	198	56	2.0	17.6	6.0	3.8	15.9	8.2	4.1	14.9	2.6
F5	4	13	15	5	54	17	0.9	14.0	6.0	3.3	5.8	7.2	2.7	12.8	3.0
F6	5	21	22	3	117	21	2.0	25.0	2.4	4.5	7.4	2.3	5.0	24.6	1.2
F7	3	8	16	4	86	10	1.3	7.0	1.9	6.1	15.6	1.3	5.7	14.3	2.3
F8	4	46	7	3	64	10	1.9	9.4	2.4	3.0	9.2	4.3	3.4	13.6	4.3
B6	5	58	11							4.2	46.3	6.7			
B10	9	130	35							3.1	55.9	3.0			

It thus appears that liming of acid soils (7A and 5A), in particular, and of soil deficient in organic matter (7B) favors the persistence of *Ps. fluorescens*, even if in diminished numbers. The effect of dried blood with and without lime was consistently beneficial, and in two cases (Palouse and 5B), alfalfa in combination with lime was also favorable. The fact that the general soil

microflora was very abundant following those treatments which were conducive to the persistence of *Ps. fluorescens* suggests the possibility that the activity of that microflora contributed to the liberation of some nutrients which could be utilized by the inoculated organism. Waksman and Lomanitz (26) found that *Ps. fluorescens* could not decompose casein but could act upon various amino acids, whereas the converse was true for *B. cereus*. The combination

TABLE 6
Influence of various treatments on the numbers of bacteria in different soils
Numbers per gram oven-dry soil, $\times 10^{-6}$

SOILS.....	PALOUSE			7A			7B			5A			5B		
	0	12	40	0	12	40	0	12	40	0	12	40	0	12	40
Incubation...days															
A1	18	7	26	0.3	0.1	0.2	7	3	2	5	8	2	5	9	8
A2	11	4	19	1.0	26.0	50.0	4	8	4	7	17	10	9	10	7
A3	17	84	39	0.5	18.0	2.4	5	50	16	10	49	10	10	65	46
A4	15	65	33	0.4	2.1	2.0	8	35	21	6	21	15	6	39	17
A5	21	33	21	0.5	5.0	0.8	3	20	10	11	20	5	14	32	17
A6	17	143	46	0.6	46.0	60.0	6	78	43	12	70	40	7	82	33
A7	21	24	28	0.7	61.0	61.0	7	56	36	11	41	16	9	47	32
A8	23	49	49	0.4	30.0	53.0	4	47	13	14	47	14	12	57	12
B6	15	240	74	0.3	250.0	107.0	6	611	72	9	81	40	6	123	41
B10	18	730	70	0.2	703.0	258.0	5	703	132	8	308	216	8	139	158

TABLE 7
Reaction (pH) of soils receiving different treatments

SOILS.....	PALOUSE	7A	7B	5A	5B
B1	6.5	4.1	5.8	4.8	5.9
B2	6.7	7.0	7.3	6.4	6.8
B3	5.4	4.4	4.6	4.3	5.0
B4	6.4	4.9	5.8	4.7	5.9
B5	5.6	4.3	6.6	4.8	6.0
B6	5.9	8.1	8.1	6.6	6.7
B7	7.2	7.1	6.4	6.3	6.2
B8	7.1	7.1	7.1	7.0	4.2
B9	6.9	7.4	7.4	7.2	6.7
B10	6.8	8.3	8.4	6.3	6.6

of the two organisms in casein media resulted in rapid decomposition of the protein by *B. cereus* and rapid ammonification of the protein derivatives by *Ps. fluorescens*. In the present investigation, counts were made also on nutrient agar; they showed large numbers of spore-forming bacilli. These may have been partly concerned in the decomposition of certain complex constituents of the organic residues to simpler compounds, which could be utilized by *Ps. fluorescens*.

Fusarium culmorum (table 8) was not appreciably affected after 12 days in the Palouse soil, regardless of the treatment, but decreased rapidly after that period. Half the treated 7A soils gave no evidence of the fungus after 40 days. The only case where the organism survived in soils 5A and 7B for 105 days was in the presence of alfalfa. There was rapid elimination of the fungus from soil 5B except where alfalfa was used; the numbers were appreciably reduced

TABLE 8
Survival of F. culmorum in soils receiving different treatments
Numbers per gram oven-dry soil, $\times 10^{-4}$

SOILS.....	PALOUSE				7A				7B				5A				5B			
Incubation...days	0	12	40	105	0	12	40	105	0	12	40	105	0	12	40	105	0	12	40	105
F1	28	36	7.0	0.1	10	16	1.0	0	77	12	0	0	45	2.9	0.1	0.1	84	2.3	0	0
F2	42	35	4.0	0.1	10	17	2.0	0	81	34	2.4	0	85	14.7	0	0	46	2.0	0	0
F3	33	19	0.2	0.3	12	8	0.0	0	67	13	2.1	1	79	18.5	0.3	0.5	45	6.7	1.8	0
F4	38	30	0.8	0.1	14	9	0.0	0	51	16	0.1	0	75	3.1	0	0	64	4.2	0	0
F5	61	51	3.0	0.0	12	6	0.0	0	108	14	0.1	0	90	2.9	0	0	81	4.2	0.01	0
F6	46	17	4.0	0.1	19	1	0.0	0	66	21	0.0	0	50	1.6	0.2	0	70	2.3	0	0
F7	33	45	3.0	0.1	20	3	0.2	0	61	20	0.1	0	60	19.5	0	0	44	4.3	0	0
F8	32	23	2.0	0.0	16	2	3.0	0	90	11	0.0	0	96	35.0	0	0	41	4.8	0.02	0

TABLE 9
Survival of Act. cellulosa in soils receiving different treatments
Numbers per gram oven-dry soil, $\times 10^{-4}$

SOILS.....	PALOUSE				7A				7B				5A				5B			
Incubation...days	0	12	40	105	0	12	40	105	0	12	40	105	0	12	40	105	0	12	40	105
A1	170	32	0	0	160	6	3	0	220	87	0.6	0.5	700	257	5	1	535	39	3.3	2.1
A2	100	38	25	0	160	52	2	0	220	76	0.5	0	810	200	5	0	328	46	2.4	1.6
A3	120	0	0	0	150	9	5	1	240	7	5.8	7.0	860	46	4	3	639	14	13.4	8.0
A4	130	1	0	0	120	13	2	0	250	41	0	0	540	200	6	2	513	38	1.0	1.2
A5	170	8	8	0	170	20	3	0	300	20	0	0	770	48	55	0	763	43	5.6	1.6
A6	130	0	0	0	120	41	23	21	230	15	0	0	450	53	14	9	391	21	2.7	1.6
A7	190	3	1	1	170	39	23	0	310	74	5.2	0	410	174	13	0	470	44	6.4	1.7
A8	160	35	0	0	180	37	9	8	330	105	5.7	0	900	408	88	0	397	49	1.4	3.2

even with this treatment, as was the case with the other soils. The pH of all soils treated with alfalfa was below 5.5, but the acid reaction alone was not responsible for the survival of this organism, since in the absence of any treatment in the acid soils (7A and 5A) the fungus also disappeared. Addition of CaCO_3 eliminated the favorable effect of the legume residue.

No correlation was found between the survival of *Act. cellulosa* (table 9) and any of the following factors: lime (pH), organic materials added, and abundance of organisms in the general soil population. The *Actinomyces*

decreased rapidly in Palouse soil and in soil 7B, but more slowly in soils 7A, 5A, and 5B. Alfalfa with lime exerted favorable effects in soils 7A and 5A; and alfalfa alone, in soils 7B and 5B. It is possible that the slower rate of disappearance of *Act. cellulosa* in soil 5A and in soil 5B was due to the heavier inocula which these soils received.

DISCUSSION

The results brought out in these experiments show that microorganisms inoculated into soil die out rapidly as a result of various unfavorable conditions of the new environment. Some organisms appear to be so incapable of adjusting themselves to their new habitat that they disappear completely, insofar as the method used is capable of demonstrating complete disappearance. Correction of certain of the factors inimical to their survival enabled them to persist, although their numbers were reduced.

Among the various factors which control biological activity in soil, reaction and food supply occupy a prominent place. A suitable reaction was found necessary for *Ps. fluorescens*; food supply was also important. With *F. culmorum* as well, food supply was an important factor.

It was previously reported (15) that various typical soil organisms, inoculated into sterile soil, increased in numbers to a maximum, then gradually decreased. It was suggested that the increase in available nutrients, as a result of sterilization, and the absence of competing organisms were responsible for these changes. The suggestion was made that the nutrient factor was probably complementary to the competition factor, since the presence of such diverse microbial forms as are encountered in soil may have resulted in a food shortage for the specific organisms; the production by the soil microflora of toxic or lytic agents active against the newly introduced forms should also be considered. The inhibition of *Azotobacter* in soils treated with K_2HPO_4 in the presence of mannite, lime, and molybdenum was partially ascribed to the antagonistic influence of the large numbers of fungi, bacteria, and actinomycetes which developed in the soils receiving the phosphate treatment (14).

Antagonism is not, however, the only important characteristic of microbial activity in soil. There are innumerable associative reactions. In this connection mention need only be made of the combined activities of various groups of organisms in the mineralization of organic nitrogenous materials in soil with the production of ammonia and its oxidation to nitrates by the nitrifying bacteria; or of the liberation of sulfur from its organic combinations and its oxidation to sulfates.

Pseudomonas fluorescens and *F. culmorum* persisted even in soils which supported very large numbers of other microorganisms. This was particularly striking in the case of the bacterium in those soils which were treated with dried blood. It was not the reaction factor alone which was responsible for the survival of *Ps. fluorescens*, since the addition of $CaCO_3$ to Palouse soil

did not lead to as great persistence of the organism as did the addition of dried blood. The possibility suggests itself that during the decomposition of the dried blood, specific nutrients were liberated which could be used by the bacterium in much the same way as *Azotobacter* used mannite or glucose as specific energy sources, despite the large numbers of organisms which developed as a result of the addition of the carbohydrate (14). The soil microflora may supply food materials to *Ps. fluorescens* by decomposing the added organic residue. The same reasoning may hold for the persistence of *F. culmorum* in soils treated with alfalfa. Recent studies by Garrett (10) may be interpreted in a similar manner. This investigator concluded that the resting mycelium of *Ophiobolus graminis* disappeared most rapidly under conditions favoring maximum microbiological activity in soil. Yet this fungus persisted in soil treated with dried blood in spite of the very marked development of the general microflora in the same soil. Garrett suggested several explanations for this phenomenon, but it seems reasonable to conclude that an additional factor was concerned, namely, that the added organic material yielded decomposition products that were utilized by the organism, which was thus enabled to survive.

The introduction of an organism into a soil involves important adjustments to the new habitat. If the organism adjusts itself partly at least as a result of soil amelioration, the inoculation experiment may be considered successful; if not, the organism will be markedly suppressed or eliminated.

SUMMARY

A number of typical soil bacteria, fungi, and actinomycetes were inoculated into five soils of varying organic matter content and pH values. All the organisms introduced into the soil decreased in number, three—*Pseudomonas fluorescens*, *Fusarium culmorum*, and *Actinomyces cellulosae*—disappearing completely.

An attempt was made to establish these three organisms in soils treated with alfalfa, straw, manure, and dried blood, with and without lime. *Pseudomonas fluorescens* usually persisted in largest numbers in soils to which dried blood or alfalfa was added. In acid soils, the correction of the reaction enabled the organism to survive. *Fusarium culmorum* survived most consistently in soils with added alfalfa but without CaCO_3 . Little correlation was noted between the persistence of *Act. cellulosae* and the treatments applied. The soils which harbored *Ps. fluorescens* and *F. culmorum* in greatest numbers were the most active microbiologically. It was suggested that these two organisms survived by virtue of their ability to utilize various decomposition products of the materials which favored their persistence.

REFERENCES

- (1) ABBOTT, E. V. 1926 Taxonomic studies on soil fungi. *Iowa State Col. Jour. Sci.* 1: 15-36.
- (2) ALLEN, M. C., AND HAENSELER, C. M. 1935 Antagonistic action of *Trichoderma* on *Rhizoctonia* and other soil fungi. *Phytopath.* 25: 244-252.

- (3) BISBY, G. R., JAMES, N., AND TIMONIN, M. 1933 Fungi isolated from Manitoba soil by the plate method. *Canad. Jour. Res.* 8: 253-275.
- (4) BISBY, G. R., TIMONIN, M. I., AND JAMES, N. 1935 Fungi isolated from soil profiles in Manitoba. *Canad. Jour. Res.* 13: 47-65.
- (5) CORDON, T. C., AND HAENSELER, C. M. 1939 A bacterium antagonistic to *Rhizoctonia solani*. *Soil Sci.* 47: 207-215.
- (6) DAINES, R. H. 1937 Antagonistic action of *Trichoderma* on *Actinomyces scabies* and *Rhizoctonia solani*. *Amer. Potato Jour.* 14: 85-93.
- (7) FRED, E. B., BALDWIN, I. L., AND MCCOY, E. 1932 Root nodule bacteria and leguminous plants. *Wisc. Univ. Studies Sci.* 5.
- (8) FRED, E. B., AND WAKSMAN, S. A. 1928 Laboratory Manual of General Microbiology. McGraw Hill Book Co., Inc., New York.
- (9) GAINES, P. L. 1930 A study of factors influencing inoculation experiments with *Azotobacter*. *Kans. Agr. Exp. Sta. Tech. Bul.* 26: 3-66.
- (10) GARRETT, S. D. 1938 Soil conditions and the take-all disease of wheat: III. Decomposition of the resting mycelium of *Ophiobolus graminis* in infected wheat stubble buried in the soil. *Ann. Appl. Biol.* 25: 742-766.
- (11) GREANEY, F. J., AND MACHACHEK, J. E. 1935 Studies on the control of root-rot disease of cereals caused by *Fusarium culmorum* and *Helminthosporium sativum*: II. Pathogenicity of *H. sativum* as influenced by *Cephalothecium roseum*, Corda, in greenhouse pot tests. *Sci. Agr.* 15: 377-386.
- (12) HINO, I. 1935 Antagonistic action of soil microbes with special reference to plant hygiene. *Trans. Third. Internatl. Cong. Soil Sci.* 1: 173-174.
- (13) JENSEN, H. L. 1931. The fungus flora of the soil. *Soil Sci.* 31: 123-158.
- (14) KATZNELSON, H. 1940 Survival of *Azotobacter* in soil. *Soil Sci.* 49: 21-35.
- (15) KATZNELSON, H. 1940 Survival of microorganisms introduced into sterilized soil. *Soil Sci.* 49: 211-217.
- (16) KHUDIAKOV, I. P. 1935 The lytic action of soil bacteria on parasitic fungi. *Microbiol. (U. S. S. R.)* 4: 193-204. (English Summary.)
- (17) LE CLERG, E. L. 1931 Distribution of certain fungi in Colorado soils. *Phytopath.* 21: 1073-1081.
- (18) LE CLERG, E. L., AND SMITH, F. B. 1928 Fungi in some Colorado soils. *Soil Sci.* 25: 433-441.
- (19) LOCHHEAD, A. G., AND THEXTON, R. H. 1936 A four-year quantitative study of nitrogen-fixing bacteria in soils of different fertilizer treatment. *Canad. Jour. Res.* 14: 166-177.
- (20) NOVOGRUDSKY, D. 1936 Use of microorganisms in control of fungal diseases of cultivated plants. *Bul. Acad. Sci. U. S. S. R. Biol. Ser.* 1: 277-293. [Abs. in *Rev. Appl. Mycol.* 16: 204, 1937.]
- (21) PAINE, F. S. 1927 Studies on the fungus flora of virgin soils. *Mycologia* 19: 248-267.
- (22) WAKSMAN, S. A. 1917 Is there any fungus flora of the soil? *Soil Sci.* 3: 565-589.
- (23) WAKSMAN, S. A. 1924 Influence of soil reaction upon the distribution of filamentous fungi in the soil. *Ecology* 5: 54-59.
- (24) WAKSMAN, S. A. 1932 Principles of Soil Microbiology, ed. 2. The Williams & Wilkins Co., Baltimore.
- (25) WAKSMAN, S. A. 1937 Associative and antagonistic effects of microorganisms: I. Historical review of antagonistic relationships. *Soil Sci.* 43: 51-68.
- (26) WAKSMAN, S. A., AND LOMANITZ, S. 1925. Contribution to the chemistry of decomposition of proteins and amino acids by various groups of microorganisms. *Jour. Agr. Res.* 3: 263-281.
- (27) WEINDLING, R. 1934 Studies on a lethal principle effective in parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other fungi. *Phytopath.* 24: 1153-1179.
- (28) WEINDLING, R., AND FAWCETT, H. S. 1936 Experiments in the control of *Rhizoctonia* damping-off of citrus seedlings. *Hilgardia* 10: 1-16.

ORIGIN AND PROPERTIES OF ALKALINE RAW HUMUS¹

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As early as 1888, Ebermayer (2) directed attention to the existence of "Alpenhumus," characterized by an accumulation of free organic remains and an alkaline reaction. At a later date, Ramann (6) described a similar type of humus, formed on calcareous outcrops in the Bavarian Alps at an elevation of about 4,000 feet. Ramann attributed the development of this type to the influence of a cool and moist climate and to the action of lime-bearing seepage water. Lang (4) has reported the occurrence of an alkaline raw humus originating from the litter of ericaceous plants above timber line in the dolomitic Alps. In spite of an alkaline reaction, this type supports cranberry, sweet broom, bilberry, and other plants found ordinarily on strongly acid soils. Wilde³ recently pointed out the occurrence of a somewhat similar type of alkaline raw humus on dolomitic limestone outcrops of Door Peninsula in northern Wisconsin. The results of a detailed investigation of this humus type are reported in this paper.

AREA OF HUMUS OCCURRENCE

Door Peninsula forms a part of the Niagara cuesta, an extensive upland bordering Lake Michigan. The Labrador ice sheet, in advancing over this formation, removed nearly all the residual soil from the higher portions and left deposits of glacial till composed chiefly of limestone material. Lacustrine clays were laid down during interglacial periods in the southern part of the peninsula. Outwash of calcareous sand was deposited in small areas.

The elevation of the peninsula ranges from about 580 to 800 feet above sea level. The topography is rolling and is marked by numerous rock outcrops, escarpments, swamps, and a few lakes.

Records of climate are available for a 32-year period at the Sturgeon Bay Station of the U. S. Weather Bureau. The mean annual temperature is

¹ Part of a thesis submitted to the faculty of the University of Wisconsin in partial fulfillment of the requirements for the degree of master of science. Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

² The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by S. A. Wilde, under whose general direction the work was done.

³ Wilde, S. A. 1937 Selection of litter, duff and humus for use in forest nurseries. Wis. Agr. Col. in cooperation with Wis. Dept. Conserv. Mimeo. Tech. Notes No. 16, Madison, Wisconsin.

42.8°F., and the mean annual precipitation is 30.3 inches. About 50 inches of snow falls from November until April. The growing season averages nearly 150 days. The mean temperature of this period is 60°F. An average of 16 inches of precipitation is well distributed during the growing season. The mean annual relative humidity at the Green Bay Station is 75 per cent. Along the shores of Green Bay and Lake Michigan the relative humidity undoubtedly averages higher, but no data for these localities are available.

The predominant soil type on the peninsula is a podzolic fine sandy loam with a mull humus (5). True podzols with a matted raw humus and a strongly cemented hardpan have developed on areas of sandy outwash. Deposits of woody peat occur in depressions. The calcareous rock outcrops have a very shallow layer of weathered material covered with raw humus of alkaline reaction, or alkaline "mor" (1).

The forest cover includes hard maple, basswood, beech, elm, white ash, red oak, balsam fir, white spruce, white cedar, and some white pine, red pine, and hemlock. The composition of forest stands and the ground cover on mull soils and podzols are typical of the Lake States region (10).

Plate 1 shows profiles of the principal types of humus occurring within the area.

MORPHOLOGICAL, CHEMICAL, AND BIOLOGICAL CHARACTERISTICS OF ALKALINE RAW HUMUS

The forest cover contributing to the development of alkaline raw humus is composed chiefly of northern white cedar (*Thuja occidentalis*) with some balsam fir (*Abies balsamea*) and incidental hardwoods. Common juniper (*Juniperus communis depressa*) occurs in openings. The ground cover is limited to the sporadic occurrence of saprophytic raw humus plants, such as *Maianthemum canadense* and *Cornus canadensis*.

A thin layer of litter is sharply delineated from the duff layer (F + H). The latter consists of dark brown matted remains of needles, leaves, and wood, chiefly those of white cedar. The total thickness of the duff varies from 4 inches on upland areas to approximately 10 inches on the lower slopes or in depressions of microrelief. In the lower portion the duff horizon grades into a narrow strip of nearly black, highly dispersed organic matter, powdery when dry and sticky when wet. This material is incorporated to some extent with weathered particles of limestone (A₁) and is underlain by an unconsolidated rock substratum.

In sampling, the undecomposed litter was removed, care being exercised to obtain the purely organic portion of the humus. The number of samples used in different analyses varied from 2 to 10, but no attempt was made to accumulate data sufficient for a statistical analysis.

The reaction, determined by means of a glass electrode, showed a variation from pH 6.7 to pH 8.0 with pH 7.5 as the weighted average. Base-exchange capacity, determined by the ammonium acetate method, varied from 98 to

TABLE 1

Average contents of phosphorus and bases in duff layer of alkaline raw humus

Per cent of oven-dry material

	P	K ₂ O	CaO	MgO
Total analysis.....	.125	.115	5.882	2.642
Soluble or replaceable fraction.....	.004	.038	2.140	0.281

TABLE 2

Proximate chemical composition of duff layers in alkaline and acid raw humus types

Per cent of dry material

CONSTITUENTS	ALKALINE RAW HUMUS	ACID RAW HUMUS
Ether-soluble fraction.....	0.31	3.63
Hot water soluble fraction.....	2.81	5.67
Alcohol-soluble fraction.....	2.47	4.94
Hemicelluloses.....	9.22	7.26
Cellulose.....	2.65	6.37
Lignin.....	38.52	43.75
Crude protein.....	14.67	11.72
Ash.....	14.46	4.55
Total accounted for.....	85.11	87.89

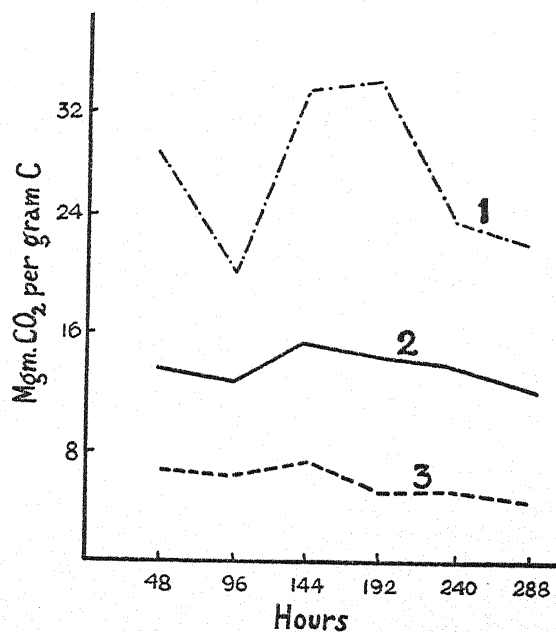


FIG. 1. RELATIVE BIOLOGICAL ACTIVITY OF DIFFERENT HUMUS TYPES AS DETERMINED BY CARBON DIOXIDE EVOLUTION

1, Mull; 2, Alkaline raw humus; 3, Acid raw humus

123 m.e. per 100 gm. Base saturation as a rule approached 100 per cent, but in some instances the content of replaceable bases exceeded base-exchange capacity because of free carbonates present in the form of calcareous dust.

The percentages of total carbon and total nitrogen averaged 34.6 and 2.17, respectively, giving a C/N ratio of 15.9.

The contents of total phosphorus, total bases, soluble phosphorus, and replaceable bases are given in table 1.

The degree of decomposition of alkaline raw humus was compared with that of an acid, hemlock raw humus (pH 4.5), collected near Chatham, Michigan. The results of fractionation analysis, according to Waksman and Stevens' methods (8, 9), are reported in table 2. The low contents of ether-soluble material and cellulose in alkaline raw humus indicate that this type occupies an intermediate position between acid raw humus and lowmoor peat (7).

As a measure of the biological activity, the carbon dioxide evolution was determined according to Heck (3) on alkaline raw humus, hardwood-hemlock duff of a mull nature, and acid raw humus. The determinations were continued for six 48-hour periods. Figure 1 illustrates the relative biological activity of these types based on the milligrams of carbon dioxide released per gram of carbon contained in the original sample.

The value of alkaline raw humus as a fertilizer has been previously reported (11).

SUMMARY

A rare type of alkaline raw humus was found on calcareous outcrops of Door Peninsula in the podzol belt of Wisconsin. This type originates chiefly from the remains of white cedar, balsam fir, and ground vegetation ordinarily associated with strongly acid soils. The dark brown, matted, peat-like duff layer varies in thickness from 4 to 10 inches. It grades into highly dispersed black organic matter, mixed with particles of limestone and underlain by a somewhat weathered rock substratum.

The chemical composition of the duff horizon is characterized by an average reaction of pH 7.5, exchange capacity of about 110 m.e. per 100 gm. with base saturation approaching 100 per cent, and a C/N ratio of 15.9. A high content of total CaO (5.9 per cent) and a low content of available phosphorus (0.004 per cent) stand out among other analytical data.

The results of fractionation analysis showed low contents of the ether-, water-, and alcohol-soluble materials and a very low content of cellulose (2.65 per cent), but rather high contents of hemicelluloses (9.22 per cent) and lignin (38.52 per cent). These data suggest that, genetically, alkaline raw humus occupies an intermediate position between acid raw humus and lowmoor peat.

In respect to biological activity, as determined by the carbon dioxide evolution method, the alkaline raw humus lies between the acid hemlock raw humus and slightly acid hardwood-hemlock duff of a mull nature.

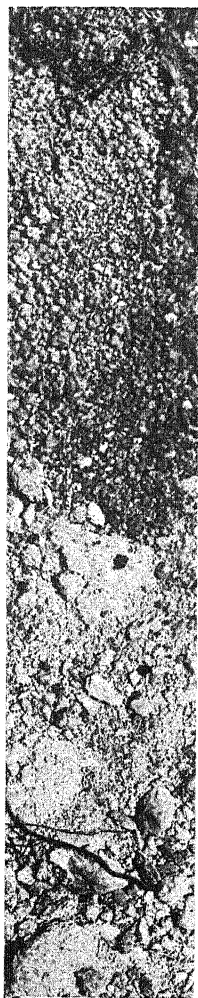
REFERENCES

- (1) BORNEBUSCH, C. H., AND HEIBERG, S. O. 1935 Report of the subcommission for forest soils. *Trans. Third Internatl. Cong. Soil Sci.* 3: 259-260.
- (2) EBERMAYER, E. 1888 Review of P. E. Müller's "Studien." *Wollny's Forsch. Geb. Agrikulturphysik* 10: 383-385.
- (3) HECK, A. F. 1929 A method for the determination of total carbon and also for the estimation of carbon dioxide evolved from soils. *Soil Sci.* 28: 225-233.
- (4) LANG, R. 1935 Zur Gliederung der Formen des Humus. *Trans. Third Internatl. Cong. Soil Sci.* 1: 368-373.
- (5) MÜLLER, P. E. 1887 Studien über die natürlichen Humusformen. Berlin.
- (6) RAMANN, E. 1911 Bodenkunde, Aufl. 3. Berlin.
- (7) WAKSMAN, S. A. 1936 Humus. The Williams & Wilkins Co., Baltimore.
- (8) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: I. Chemical nature of organic complexes in peat and methods of analysis. *Soil Sci.* 26: 113-137.
- (9) WAKSMAN, S. A., AND STEVENS, K. R. 1930 A critical study of the methods for determining the nature and abundance of soil organic matter. *Soil Sci.* 30: 97-116.
- (10) WILDE, S. A. 1933 The relation of soils and forest vegetation of the Lake States Region. *Ecology* 14: 94-105.
- (11) WILDE, S. A., BURAN, S. F., AND GALLOWAY, H. M. 1937 Nutrient content and base exchange properties of organic layers of forest soils in the Lake States region. *Soil Sci.* 44: 231-239.

PLATE 1

PROFILE CHARACTERISTICS OF THREE HUMUS TYPES FOUND IN DOOR
PENINSULA, WISCONSIN

1. Crumb mull on a slightly podzolized silt loam; 2. Acid raw humus on a podzol sandy loam with ortstein horizon; 3. Alkaline raw humus underlain by limestone bedrock. The monoliths represent sections 20 inches deep.



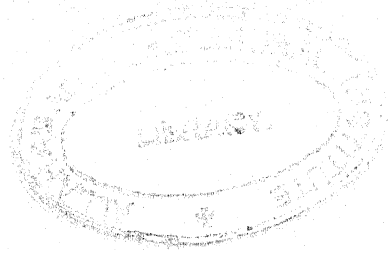
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AVAILABILITY OF FIXED POTASSIUM TO PLANTS¹

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It has been shown by many workers (2, 4, 5, 7) that when soluble potash salts are applied to soils, a considerable part of the potassium is soon taken up by the soil colloids and held in an exchangeable form readily available to plants. In time, however, a large part of this added potassium is apparently transformed (3, 6, 13, 14, 16, 18, 21) into a nonexchangeable form difficultly available to plants. This potassium, so fixed that it cannot be replaced by ordinary methods of removing exchangeable ions, such as washing with neutral salt solutions and electrodialysis, will be referred to hereinafter as "fixed potassium."

Experiments using permutite saturated with various cations as a substrate for plant growth were first reported by Nostitz (17). At approximately the same time, Joffe and McLean (15) used, in a similar manner, soils saturated with several cations. Gedroiz (5) and Joffe (12) more recently made extensive use of soils saturated with various ions as mediums for experimental studies of plant growth. Jenny and Cowan (9), Horner (8), Albrecht and McCalla (1), and Jenny and Overstreet (10) have studied the use of colloidal clay materials as nutrient substrates for plants. Recent work to account for the mechanism of potassium fixation has been reported, but little is known of the relative degree of availability to plants of fixed potassium. Indirect methods (3, 19) have been used, but it is thought that this paper constitutes the first report of an attempt to investigate directly the degree of availability to plants of fixed potassium.

EXPERIMENTAL PROCEDURE

This problem was investigated by growing tomato plants in sand cultures in some of which potassium fixed by bentonite was used as the sole source of this element. For comparison, other sources of potassium were used, including exchangeable potassium, potassium of untreated bentonite, and soluble potassium salts. Nutrients other than potassium were supplied as soluble salts. The growth responses, green and dry weights, and the potassium contents of the plants in each treatment were determined as evidences of potassium availability.

¹ Journal Series paper of the New Jersey Agricultural Experiment Station, department of plant physiology.

The potassium in a fixed condition was prepared as follows: 75-gm. samples of Wyoming bentonite were treated with excess potassium chloride and then alternately dried at 100°C. and wetted. The excess potassium chloride was removed by treating each sample with 2,000 cc. of neutral (pH 7.0) normal calcium acetate. The samples were then treated with 200 cc. of normal calcium chloride and washed free of chloride. There then remained in the bentonite 9.2 m.e. of potassium per 100 gm. of the sample in fixed or unexchangeable condition. Calcium was used as the final replacing ion, since work by Gedroiz (5) indicated that plants were not injured in the presence of clays saturated with large quantities of calcium ions, as they were when clays saturated with some of the other common cations were used.

To furnish potassium in exchangeable form, separate 75-gm. samples of bentonite were washed with approximately 2,500 cc. of a neutral (pH 7.0) normal solution of potassium acetate followed by treatment with 200 cc. of a normal potassium chloride solution. The samples were then washed with distilled water until free of chlorides. As determined by the ammonium acetate procedure, the exchangeable potassium content of the bentonite samples so prepared was 76.6 m.e. per 100 gm. of sample.

Two experiments were conducted simultaneously. They were essentially similar with respect to the nutrient treatments employed, but differed with respect to the manner in which the nutrient solutions were supplied to the cultures.

In the first experiment, the nutrient solution was applied to the surface of the sand by the constant drip method of solution renewal after the manner of Shive and Stahl (20). Twenty-seven cultures were employed, comprising twelve different treatments. Each of the bentonite materials tested was used in concentrations of 1 and 2 per cent of the weight of the sand in each culture. In certain cultures no bentonite materials were used. Table 1 gives the plan of this experiment. Each culture consisted of a 6-inch nonporous porcelain glazed pot with 1,600 gm. of white quartz sand in which was incorporated the bentonite sample. The incorporation of the bentonite in the sand was done by mixing the sample with a sufficient quantity of the nutrient solution to form a thick but fluid suspension, which was then thoroughly mixed with the sand. The drainage hole in the bottom of the pot was covered with a small watch glass and then with 250 gm. of washed fine quartz gravel to facilitate free drainage. The sand employed was thoroughly washed with water and successively leached with 5 per cent hydrochloric acid, with 5 per cent ammonium hydroxide, and with distilled water.

A total of 11,300 cc. of nutrient solution was supplied to each culture during the experimental period, which extended from December 17, 1937 to January 24, 1938. Table 2 gives the composition of the solutions employed. In order to determine the availability of potassium from the bentonite materials, a quantitative balance sheet was kept of all potassium added to and recovered from one culture in each of the twelve different cultural treatments. These are designated as S or "standard cultures."

TABLE 1

Experimental treatments of cultures grown with the constant drip method of continuous solution supply and the total amount of excess solution escaping from each standard culture during the experiment

CULTURE	TREATMENT	NUTRIENT SOLUTION	EXCESS SOLUTION
			cc.
1 S	1 per cent untreated bentonite—with plant	—K	10,435
19	1 per cent untreated bentonite—with plant	—K
2 S	2 per cent untreated bentonite—with plant	—K	10,170
20	2 per cent untreated bentonite—with plant	—K
3 S	1 per cent fixed K bentonite—without plant	—K	10,300
4 S	1 per cent fixed K bentonite—with plant	—K	10,580
21	1 per cent fixed K bentonite—with plant	—K
22	1 per cent fixed K bentonite—with plant	—K
5 S	2 per cent fixed K bentonite—without plant	—K	10,275
6 S	2 per cent fixed K bentonite—with plant	—K	10,350
23	2 per cent fixed K bentonite—with plant	—K
24	2 per cent fixed K bentonite—with plant	—K
7 S	1 per cent exchangeable K bentonite—without plant	—K	10,560
8 S	1 per cent exchangeable K bentonite—with plant	—K	10,220
25	1 per cent exchangeable K bentonite—with plant	—K
26	1 per cent exchangeable K bentonite—with plant	—K
9 S	2 per cent exchangeable K bentonite—without plant	—K	10,190
10 S	2 per cent exchangeable K bentonite—with plant	—K	10,090
27	2 per cent exchangeable K bentonite—with plant	—K
11 S	Sand only—with plant	—K	10,330
16	Sand only—with plant	—K
17	Sand only—with plant	—K
18	Sand only—with plant	—K
12 S	Sand only—with plant	+K	9,990
13	Sand only—with plant	+K
14	Sand only—with plant	+K
15	Sand only—with plant	+K

TABLE 2

Compositions of nutrient solutions expressed in volume molecular concentrations

NUTRIENT SOLUTION SUPPLY	TREATMENT	MgH ₂ (PO ₄) ₂	Ca(NO ₃) ₂	MgSO ₄	MgO	K ₂ SO ₄
Constant drip (Experiment 1)	{ Complete solution	.00225	.00450	.00225	.00083	.001125
	{ Minus potassium	.00225	.00450	.00225	.00083
Autoirrigation (Experiment 2)	{ A Complete solution	.00008	.00125	.00010	.00003	.000450
	{ Minus potassium	.00008	.00125	.00010	.00003
	{ B Complete solution	.00060	.01000	.00080	.00022	.001800
	{ Minus potassium	.00060	.01000	.00080	.00022

In the second experiment the nutrient solution was supplied automatically from a reservoir by means of porous clay autoirrigator cups "planted" in the sand in the pots and at a rate determined by the loss of water by transpiration from the plants. The orifices of the cups were fitted into rubber stoppers and connected by glass tubing to the nutrient solution reservoir slightly below the level of the cultures. The tension produced by the withdrawal of water from the sand by the plants caused the movement of the solution into the cultures. These are termed "autoirrigated cultures." The sand-bentonite mixtures were prepared in the same manner as were those in the first experiment except that 2 per cent mixtures were employed throughout. The drainage holes of the pots were tightly closed with rubber stoppers. Evaporation from the surface of the sand was prevented by covering the pots with circular discs of paraffined cardboard fitted carefully around the stems of the plants and fastened to the pots.

Table 2 gives the composition of the nutrient solutions which were used. This experiment extended from December 16, 1937 to February 21, 1938. Solution A was used until February 3, when it was replaced by solution B to provide a greater supply of nutrient ions.

Except for one treatment in each experiment where a complete nutrient solution was used, nutrient solutions lacking potassium were used throughout. To all solutions when applied were added sufficient quantities of boric acid, manganese sulfate, and ferrous sulfate to provide a concentration of $\frac{1}{4}$ p.p.m. each of boron, manganese, and iron.

Small 2-week-old Marglobe tomato seedlings selected for uniformity were used in both experiments. Systematic seed selection from the progeny of a single plant over a period of several years, in this laboratory, made for a high degree of plant uniformity.

At harvest, the green and dry weights of the tops and roots of the plant in each culture were obtained. Considerable care was taken to free the roots from sand as far as possible. The weights of the roots were corrected when necessary for the weight of the sand found in the sample after drying.

The potassium was determined as the cobaltinitrite (22).

PRESENTATION OF DATA

Constant drip method of solution renewal

The loss of potassium from each of the standard cultures by way of the escaping solution is given in table 3. The amounts of the escaping solutions are given in table 1. Although a solution lacking potassium was used in all the treatments in which bentonite materials were used, the effect of the solution in releasing potassium from the variously prepared bentonite samples varied greatly. From culture 1 S to 6 S inclusive no potassium was released, with the exception of a trace from treatment 2 S. This indicates that the culture solutions did not displace the potassium either from the untreated bentonite or

from the bentonite containing fixed potassium. No potassium was found in the solution escaping from treatment 11 S which was a control culture containing no bentonite or other added potassium.

A large proportion of the potassium originally present in cultures 7 S, 8 S, 9 S, and 10 S, containing exchangeable potassium, was displaced by the solution and escaped, as shown in table 3. The most rapid loss of this potassium occurred during the first two weeks of the experiment, when more than nine-tenths of the total loss took place. Of the 480 mgm. originally present in exchangeable form in culture 7 S, 417.8 were displaced from the culture. Of the 960 mgm. present in culture 9 S, 911.2 were displaced and recovered in the drip. The absorption of potassium by the plants in cultures 8 S and 10 S accounts for the somewhat lower loss of potassium from these cultures than from cultures 7 S and 9 S respectively without plants.

TABLE 3

Potassium content of accumulated excess solutions escaping from each of the standard cultures in experiment 1 collected on the indicated dates*

STANDARD CULTURES	2 S	7 S	8 S	9 S	10 S	12 S
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Dec. 23	Trace	277.1	263.9	463.3	512.5	159.6
Dec. 29	117.2	104.2	336.7	308.9	126.8
Jan. 3	21.1	25.2	55.0	47.8	96.6
Jan. 10	2.4	10.5	47.1	32.2	126.0
Jan. 15	9.1	1.7	124.5
Jan. 24	Trace	Trace	153.9
Totals.....	Trace	417.8	403.8	911.2	903.1	787.4

* No potassium was found in the solution escaping from culture 1 S, 3 S, 4 S, 5 S, 6 S, or 11 S.

Potassium added as a constituent of the complete solution passed through culture 12 at a fairly uniform rate during the experimental period, as shown in table 3. Of the 901.9 mgm. of potassium added, 787.4 were recovered in the excess drip from this culture. Table 4 presents the balance sheet of this culture with the additions in amounts of solution and potassium added and recovered during the experiment. The amount of potassium passing through the culture represented 87 per cent of the total of 901.9 mgm. of potassium supplied to the culture during the experiment. The largest part of this difference, amounting to 93.1 mgm. of potassium, was accounted for by the absorption of potassium by the plant. The balance of 21.4 mgm. of potassium remained in the culture.

It will be observed (table 5) that the green and dry weights and the potassium contents of plants grown with untreated bentonite and with fixed potassium were lower than those of the plants grown with the bentonite containing ex-

TABLE 4

Balance sheet showing the quantities of complete solution and the amount of potassium added to and recovered from culture 12 S at intervals throughout the experiment

DATE	SOLUTION ADDED	POTASSIUM ADDED IN SOLUTION	DRIp PASSING THROUGH CULTURE	POTASSIUM RECOVERED IN DRIp
	cc.	mgm.	cc.	mgm.
Dec. 18	1,750	145.8
Dec. 21	960	80.0
Dec. 23	600	50.0	1,900	159.6
Dec. 27	480	40.0
Dec. 29	1,750	142.8	1,580	126.8
Jan. 3	1,120	91.4	1,230	96.6
Jan. 8	1,300	106.1
Jan. 10	1,500	126.0
Jan. 13	360	29.4
Jan. 15	1,750	136.2	1,500	124.5
Jan. 17	610	39.7
Jan. 21	620	40.5
Jan. 24	2,280	153.9
Total.....	11,300	901.9	9,990	787.4

TABLE 5

Green and dry weights and potassium contents of tomato plants grown in experiment 1 with the constant drip method of solution renewal

CULTURE	TREATMENT	GREEN WEIGHT	DRY WEIGHT				K CONTENT OF DRY WEIGHT OF WHOLE PLANT	TOTAL K AB- SORBED BY WHOLE PLANT
		Tops	Tops	Roots	Total			
		gm.	gm.	gm.	gm.	per cent	mgm.	
1 S	1 per cent untreated bentonite	0.491	0.0855	0.0120	0.0975	0.11	0.09	
19	1 per cent untreated bentonite	0.744	0.1277	0.0139	0.1416	0.26	0.33	
2 S	2 per cent untreated bentonite	1.206	0.1746	0.0135	0.1881	0.19	0.34	
20	2 per cent untreated bentonite	4.450	0.5825	0.0380	0.6205	0.33	1.91	
4 S	1 per cent fixed K bentonite	0.373	0.0745	0.0127	0.0872	0.12	0.09	
21	1 per cent fixed K bentonite	0.572	0.1023	0.0199	0.1222	0.25	0.26	
22	1 per cent fixed K bentonite	0.809	0.1377	0.0217	0.1594	0.26	0.36	
6 S	2 per cent fixed K bentonite	0.597	0.1097	0.0178	0.1275	0.35	0.38	
23	2 per cent fixed K bentonite	0.721	0.1289	0.0175	0.1464	0.22	0.28	
24	2 per cent fixed K bentonite	0.738	0.1151	0.0191	0.1342	0.36	0.41	
*8 S	1 per cent exchangeable K bentonite	8.150	0.9801	0.1400	1.1201	1.06	10.37	
25	1 per cent exchangeable K bentonite	4.300	0.5807	0.0846	0.6653	0.73	4.22	
26	1 per cent exchangeable K bentonite	2.550	0.3500	0.0589	0.4089	0.93	3.24	
10 S	2 per cent exchangeable K bentonite	5.000	0.6302	0.0108	0.6410	0.69	4.34	
*27	2 per cent exchangeable K bentonite	15.500	1.4712	0.2183	1.6895	2.96	43.61	
11 S	Sand only -K	0.488	0.0832	0.0187	0.1019	0.17	0.14	
16	Sand only -K	0.540	0.1016	0.0251	0.1267	0.21	0.21	
17	Sand only -K	0.472	0.0914	0.0185	0.1099	0.10	0.09	
18	Sand only -K	0.396	0.0793	0.0230	0.1023	0.11	0.09	
12 S	Sand only +K	24.600	1.9428	0.2908	2.2336	4.79	93.10	
15	Sand only +K	25.150	1.8216	0.2553	2.0769	4.91	89.44	

* See text concerning these cultures.

changeable potassium. Neither the untreated bentonite nor the bentonite containing fixed potassium provided available sources of potassium for the plants in these cultures. Figure 1, plate 1, shows representative plants in each treatment.

Where exchangeable potassium was used, the plants made considerably more growth and absorbed more potassium than where fixed potassium was used. The results indicate that the potassium present in exchangeable form was readily available to the plants and that its displacement from the cultures by the nutrient solution during the first part of the experiment limited the growth of the plants in these cultures during the latter part of the experiment. This conclusion is borne out by the growth of the plants in culture 27. Early in the experiment the drainage system of this culture became temporarily clogged, and loss of the bentonite material by leaching was materially retarded compared with that of the other cultures containing exchangeable potassium. The plant in this culture absorbed 43.61 mgm. of potassium during the experiment, compared with an absorption of only 4.34 mgm. by the plant in culture 10 S, which received the same nutrient treatment but in which free drainage occurred. A similar situation occurred but to a less marked degree in culture 8 S. The relatively high potassium contents of the plants in cultures 27 and 8 S are thus accounted for.

Autoirrigation method of solution supply

Plants grown in the autoirrigated cultures responded somewhat differently from those supplied with solutions dripped on the surface of the sand. Figure 2, plate 1, shows the appearance on January 21, of one culture of each treatment in the autoirrigated experiment. Table 6 gives the green and dry weights and potassium contents of the tissues at harvest. The greatest growth and potassium absorption occurred in the cultures supplied with exchangeable potassium and with complete solution. The exchangeable potassium in cultures 5 and 6 was readily available and was rapidly absorbed by these plants. This fact was emphasized by their high potassium contents, which were 228.5 and 250.8 mgm. respectively, exceeding the potassium contents of the plants in cultures 7 and 8, which were supplied with a complete nutrient solution. The fact that the potassium contents of plants supplied with a complete solution were lower than those of plants supplied with exchangeable potassium is accounted for by the very low nutrient level of the solution used originally in the latter cultures. As noted previously, the nutrient level was increased on February 3 to allow a rate of growth more nearly corresponding to that obtained under the usual conditions of nutrient solution renewal. No potassium was lost from the autoirrigated cultures by reason of the nature of the system of solution supply, in contrast with the loss of exchangeable potassium in the first experiment. The presence of exchangeable potassium in the culture throughout the experiment accounts for the high content of this element in the plant tissues.

Root development in the autoirrigated cultures was excellent. Figure 3, plate 1, shows the comparative root development in the autoirrigated cultures, and in those where the continuous solution renewal method was used. The roots in the autoirrigated cultures were not so numerous or so slender as those in the cultures supplied with solution continuously dripped on the surface of the sand, but they appeared to be healthy and capable of functioning actively.

Plants in cultures 1 and 2 absorbed the potassium of the untreated bentonite to some extent. At harvest their green and dry weights were comparable to those of cultures 7 and 8 grown with complete nutrient solution, although their potassium contents were much lower.

TABLE 6
Green and dry weights and potassium contents of tomato plants grown in autoirrigated sand cultures

CULTURE	TREATMENT	NUTRIENT SOLUTION	GREEN WEIGHT	DRY WEIGHT				K CONTENT OF DRY WEIGHT OF WHOLE PLANT	TOTAL K ABSORBED BY WHOLE PLANT
			Tops	Tops	Roots	Total			
			gm.	gm.	gm.	gm.	per cent		mgm.
1	2 per cent untreated bentonite	-K	46.50	5.1250	1.0520	6.1770	0.69		35.23
2	2 per cent untreated bentonite	-K	37.00	3.7800	0.9252	4.7052	0.44		16.55
3	2 per cent fixed K bentonite	-K	13.50	1.7382	0.3771	2.1153	0.44		7.65
4	2 per cent fixed K bentonite	-K	13.00	1.7336	0.4138	2.1474	0.50		8.67
5	2 per cent exchangeable K bentonite	-K	47.50	5.5891	1.2012	6.7903	4.09		228.48
6	2 per cent exchangeable K bentonite	-K	41.50	4.7518	0.7499	5.5017	5.28		250.80
7	Sand only	+K	41.00	5.4386	1.6671	7.1057	1.77		96.26
8	Sand only	+K	35.90	4.9166	1.7226	6.6392	2.06		101.09
9	Sand only	-K	2.50	0.4308	0.1103	0.5411	0.54		2.32
10	Sand only	-K	3.05	0.4969	0.1026	0.5995	0.38		1.88

Plants in cultures 9 and 10, supplied with solution lacking potassium, were found to contain a total of only 2.32 and 1.88 mgm. of potassium respectively. An undetermined portion of this quantity of potassium was present in the seedling when the experiment was started. The remainder was apparently present as an impurity in the culture or nutrient salts used.

The green and dry weights of plants in cultures 3 and 4 supplied with fixed potassium were lower than those of plants supplied with any other source of potassium. The potassium contents of the plants in the two cultures of this treatment were 7.65 and 8.67 mgm. per plant respectively. The difference between the potassium content of these plants and those in cultures 9 and 10 grown without potassium and which averaged approximately 2 mgm. per plant leaves a difference of approximately 6 mgm. per plant which was absorbed from the 115 mgm. of fixed potassium originally present. Since no potassium was

detected in the excess solution lost from the cultures containing fixed potassium when the nutrient solution was continuously dripped through the sand, it may be concluded that the fixed potassium in cultures 3 and 4 (table 6) was made available to the roots to a slight extent as a result of some specific interaction of the root and the substrate. The excretion of carbonic acid by the plant roots followed by an acid hydrolysis of the clay complex may possibly account for the consequent release of fixed potassium to a slight degree.

The recent theory of contact exchange proposed by Jenny and Overstreet (11) for the mechanism to account for the absorption of adsorbed cations from bentonite clay materials might well explain the release of fixed potassium to plant roots. The plants were able to absorb considerably more potassium from the bentonite material both untreated and containing fixed potassium than was chemically exchangeable by the methods employed for ion displacement with these materials.

It is interesting to note from the differences between the potassium absorbed by plants from cultures containing untreated bentonite and those containing fixed potassium (table 6) that the intermittent drying and wetting coincident with the preparation of the fixed potassium apparently converted a part of the potassium in the untreated bentonite to a fixed form not so readily available to the plants.

SUMMARY

Tomato plants were grown in sand cultures to study the availability of fixed potassium as compared with exchangeable potassium and to compare the availability of these sources of potassium with that present in untreated bentonite and as a constituent of a complete nutrient solution. Fixed potassium consisted of that potassium undisplaceable by calcium ions after the alternate wetting and drying of potassium-saturated Wyoming bentonite by the methods described. Exchangeable potassium was prepared by saturation of the untreated bentonite with potassium by the usual methods. The following is a summary of the more important results:

Fixed potassium was utilizable by tomato plants to but a slight extent for growth.

Fixed potassium was not nearly so readily available to tomato plants as was exchangeable potassium or the potassium of untreated bentonite.

Fixed potassium became available to the tomato plants to a slight extent apparently by means of root action, perhaps either by carbonic acid excretion or by means of direct contact exchange between roots and the bentonite sample containing this form of potassium.

REFERENCES

- (1) ALBRECHT, W. A., AND MCCALLA, T. M. 1938 The colloidal clay fractions of soil as a cultural medium. *Amer. Jour. Bot.* 25: 403-406.
- (2) BARTHOLOMEW, H. P., AND JANSSEN, G. 1931 The rate of absorption of potassium by plants and its possible effect upon the amount of potassium remaining in soils from applications of potassium fertilizers. *Ark. Agr. Exp. Sta. Bul.* 265.
- (3) CHAMINADE, R. 1936 La retrogradation du potassium dans les sols. *Ann. Agron.* 6: 818-830.

- (4) ENFIELD, G. H., AND CONNER, S. D. 1936 The fixation of potassium by muck soils. *Jour. Amer. Soc. Agron.* 28: 146-155.
- (5) GEDROIZ, K. K. 1931 Exchangeable cations of the soil and the plant: I. Relation of plant to certain cations fully saturating the soil exchange capacity. *Soil Sci.* 32: 51-63.
- (6) HARRIS, H. C. 1937 Effect of lime on the availability and the fixation of potassium in soils. *Soil Sci.* 44: 265-275.
- (7) HOAGLAND, D. R., AND MARTIN, J. C. 1935 Absorption of potassium by plants and fixation by the soil in relation to certain methods for estimating available nutrients. *Trans. Third Internatl. Cong. Soil Sci.* 1: 99-103.
- (8) HORNER, G. M. 1936 Relation of the degree of base saturation of a colloidal clay by calcium to the growth, nodulation and composition of soybeans. *Missouri Agr. Exp. Sta. Res. Bul.* 232.
- (9) JENNY, H., AND COWAN, E. W. 1933 The utilization of adsorbed ions by plants. *Science* 77: 394-396.
- (10) JENNY, H., AND OVERSTREET, R. 1938 Contact effects between plant roots and soil colloids. *Proc. Natl. Acad. Sci.* 24: 384-392.
- (11) JENNY, H., AND OVERSTREET, R. 1939 Cation interchange between plant roots and soil colloids. *Soil Sci.* 47: 257-272.
- (12) JOFFE, J. S. 1935 Behavior of replaceable cations in the soil and their availability. *Trans. Third Internatl. Cong. Soil Sci.* 1: 66-67.
- (13) JOFFE, J. S., AND KOLODNY, L. 1937 Fixation of potassium in soils. *Soil Sci. Soc. Amer. Proc.* (1936) 1: 187-192.
- (14) JOFFE, J. S., AND KOLODNY, L. 1938 The distribution and fixation of potassium in the profile of brown podzolic soils and sand podzols. *Soil Sci. Soc. Amer. Proc.* (1937) 2: 239-241.
- (15) JOFFE, J. S., AND McLEAN, H. C. 1927 Availability of replaceable cations. *Proc. First Internatl. Cong. Soil Sci. Soc. Comm.* 2: 256-263.
- (16) MORGAN, M. F. 1935 Changes in exchangeable bases in soils as related to fertilizer applications, leaching and crop removal. *Trans. Third Internatl. Cong. Soil Sci.* 1: 70-72.
- (17) NOSTITZ, A. V. 1925 Zur Bedeutung der basisch austauschbaren Bodennährstoffe für die Pflanzen und über Einwirkung des Kalkes auf die absorbierenden Bodenkörper. *Landw. Vers. Sta.* 103: 159-177.
- (18) PAGE, H. J., AND WILLIAMS, W. 1925 Studies in base exchange in Rothamsted soils. *Trans. Faraday Soc.* 20: 573-585.
- (19) SCHACHTSCHABEL, P. 1937 Aufnahme von nicht-austauschbaren Kali durch die Pflanzen. *Bodenk. u. Pflanzenernähr.* 3 (48) H 1/2: 107-133.
- (20) SHIVE, J. W., AND STAHL, A. L. 1927 Constant rates of continuous solution renewal for plants in water cultures. *Bot. Gaz.* 84: 317-323.
- (21) VOLK, N. J. 1934 The fixation of potash in difficultly available form in soil. *Soil Sci.* 37: 267-287.
- (22) WILCOX, L. V. 1937 Determination of potassium. *Indus. and Engin. Chem. Analyt. Ed.* 9: 136-138.

PLATE 1

UTILIZATION OF POTASSIUM BY TOMATO PLANTS

FIGS. 1 AND 2. Comparative utilization by tomato plants of potassium from different nutrient sources: nutrient solutions applied by the constant drip method (fig. 1) and by autoirrigation (fig. 2). Treatments, left to right, minus potassium, 2 per cent fixed potassium, 2 per cent untreated bentonite, 2 per cent exchangeable potassium, complete nutrient solution.

FIG. 3. Root systems of tomato plants growing in sand cultures supplied with complete solution by the constant drip method (left), and by autoirrigation (right).



FIG. 1

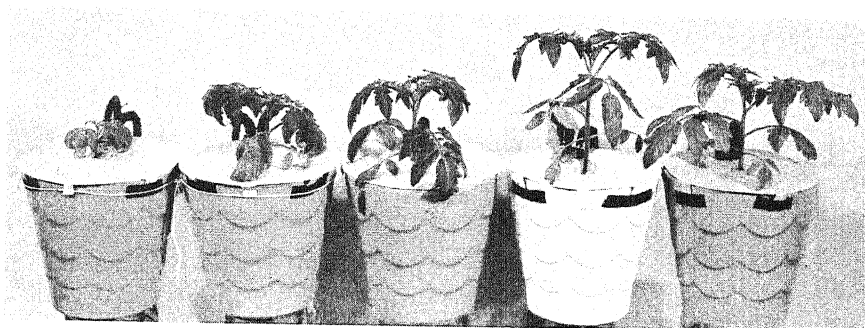


FIG. 2

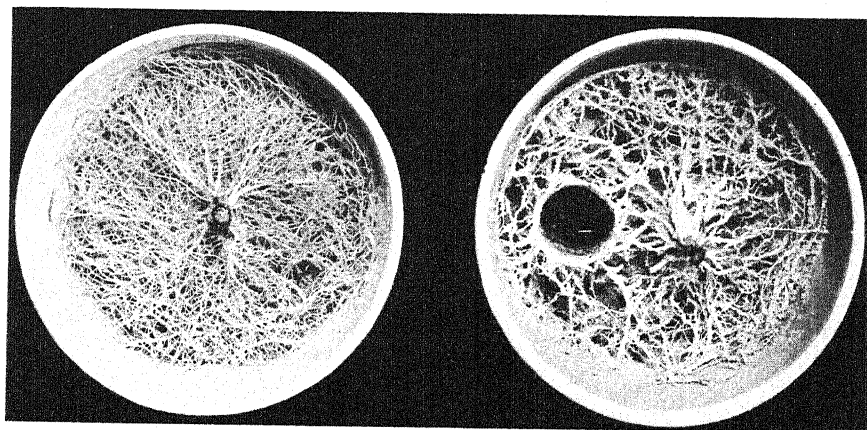


FIG. 3

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THE ROLE OF POTASSIUM IN PLANTS: II. EFFECT OF VARYING AMOUNTS OF POTASSIUM ON THE GROWTH STATUS AND METABOLISM OF TOMATO PLANTS¹

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A preliminary experiment by the author (15) in which tomato plants were grown in sand culture with 0, 45, 175, and 375 p.p.m. of potassium gave results that indicated that 45 p.p.m. was the optimum solution. Above this point growth and carbohydrate production seemed to decrease slowly, and below this point (i.e., with 0 p.p.m. of potassium) they decreased rapidly. In this preliminary investigation, the plants were not allowed to set fruit. Moreover, there was no evidence that 45 p.p.m. of potassium was the minimum amount of potassium which would produce normal growth under the experimental conditions.

EXPERIMENTAL METHODS

The scope of this investigation was extended, therefore, to include a number of solutions which would contain amounts of potassium between 0 and 45 p.p.m. In these experiments the plants were allowed to set fruit in order to approximate more closely field conditions. In view of the contradictory statements in the literature on the effects of potassium on plant metabolism, the nitrogenous, carbohydrate, and mineral metabolism of these plants was studied. The experiment was so planned that a progressive series of potassium-deficient plants would be obtained and that the effects of this progressive deficiency would be reflected in the metabolism. In this way, the effects of potassium on plant metabolism could be followed more accurately.

Young tomato seedlings of the Rutgers variety which had been grown in pots containing good loam soil and selected for uniformity were washed free of soil and transplanted to glazed crocks containing washed white quartz sand. By means of a constant drip method, each crock was supplied with $3\frac{1}{2}$ liters of nutrient solution every 24 hours.

The plants were set in sand February 22, 1937. At this time they were

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rather hard and contained abundant starch. They received no nutrient solution for 1 week, and at the end of this period resembled typical minus-nitrogen plants. The seedlings were then divided into seven series, to which 0, 2.5, 5, 11, 22, 45, and 175 p.p.m. of potassium were given respectively as K_2SO_4 . Two plants were placed in a pot, 10 pots constituting a series. Five of these pots were then placed on the side of the greenhouse receiving the most light, and the remaining five pots were placed for the time being on the side receiving inferior light.

The composition of the nutrient solutions is given in tables 1 and 2.

TABLE 1
Partial volume molecular concentrations of the nutrient solutions

SERIES	NaH_2PO_4	K_2SO_4	$Ca(NO_3)_2$	$MgSO_4$
1	.0045	.00000	.0090	.0045
2	.0045	.00004	.0090	.0045
3	.0045	.00008	.0090	.0045
4	.0045	.00015	.0090	.0045
5	.0045	.0003	.0090	.0045
6	.0045	.0006	.0090	.0045
7	.0045	.0023	.0090	.0045

TABLE 2
Parts per million of ions in the nutrient solution

SERIES	K	Na	Ca	Mg	NO_3 AS N	PO_4 AS P	SO_4 AS S
1	0	103.5	360	108	252	139.5	162
2	2.5	103.5	360	108	252	139.5	163
3	5	103.5	360	108	252	139.5	164
4	11	103.5	360	108	252	139.5	166
5	22	103.5	360	108	252	139.5	170
6	45	103.5	360	108	252	139.5	177.5
7	175	103.5	360	108	252	139.5	206

The nutrient solutions differ only in potassium and sulfur content. Since it has been shown that the sulfate ion in concentrations above deficiency levels has little effect on plant metabolism (1), it may be assumed that potassium is the only limiting nutritional factor in this experiment. The growth and appearance of the plants in all the series were noted at regular intervals. Microchemical tests for potassium and starch were also frequently conducted. Determinations of reducase activity were made, but yielded negative results in all cases.

Half of the plants were harvested April 1, 1937, 38 days after being set in sand; the remaining plants were harvested May 24, 1937, 91 days after being set in sand. The times of harvesting were determined by the inception of "early" and "extreme" potassium deficiency symptoms in the minus and low

potassium treatments. At both harvests, the heights of the plants and the fresh weights of the stems were determined. At the second harvest, ripe fruits were collected and weighed and their diameters taken. The fruits were kept in cold storage for one to two weeks before being analyzed.

After the growth data were obtained, the plants were harvested and immediately prepared for analysis. The plants were cut in half, and four fractions, upper and lower blades and upper and lower stems, were taken for analysis. The leaf material was well minced, and the stems were sliced into small pieces. A portion of the plant material was dried, as recommended by Link (8), and used for the determination of total nitrogen, carbohydrate, and mineral fractions.

For the determination of soluble nitrogen fractions, 100- to 200-gm. aliquots of the fresh plant material were extracted with boiling water according to the procedure of Davidson and Shive (3). This procedure will hydrolyze the amide glutamine (14), and the glutamine nitrogen would be found in the ammonia fraction. Glutamine, however, is comparatively low in tomatoes where nitrates are the source of nitrogen (2). Total soluble nitrogen, nitrate N, ammonia N, amide N, and amino N were determined as described by Nightingale et al. (10).

The dried material was ground to a fine powder in a ball mill. Total nitrogen, including nitrates, was determined by the Kjeldahl method on 0.5-gm. samples. Protein N was calculated as the difference between total N and total soluble N. Soluble organic N was calculated as the difference between total soluble N and nitrate N.

Carbohydrate determinations were conducted on 0.5- to 1-gm. samples of the dried material. The samples were extracted with alcohol, and the alcohol extract was cleared as described by Nightingale et al. (10). For the determination of reducing sugars, 5-cc. aliquots of the cleared extract were used. Other 5-cc. aliquots were inverted with invertase, and total sugars determined. The determination of the reducing power was carried out by the method of Van der Plank (13).

In the case of the fruits, aliquots of the hot water extracts were used for the determination of reducing sugars, total sugars, and fructose. The aliquots were cleared and treated in the same manner as the extracts of dried material.

In all cases glucose was calculated as the difference between total reducing sugars and fructose, and sucrose as the difference between total sugars and total reducing sugars.

The insoluble residue remaining after the alcohol extraction, was treated for starch and hemicellulose, as described by Nightingale et al. (10). The reducing power was determined on 5-cc. aliquots by the method of Van der Plank (13).

For the mineral determinations, 0.5- to 1-gm. samples were ashed in a muffle furnace at 600°C., and the ash was weighed. The ash was dissolved in 1-4 HCl, and potassium, sodium, calcium, magnesium, and phosphates were determined (16).

RESULTS

Effect of nutrient treatments on plant growth

After receiving nutrient solution for 14 days, series 1 and 2 began to show the initial symptoms of potassium deficiency. Many of these plants at this time were stunted, hard, and chlorotic and in general displayed all the symptoms of "early" potassium deficiency that were discussed in a previous paper by the author (15). Series 3 and 4 began to show similar symptoms after receiving the nutrient solution 20 days. Series 5 began to show slight symptoms, as manifested by mottling of the leaves and a light green color, 27 days after receiving the nutrient solution. Series 6 and 7 showed no deficiency symptoms at the time of the first harvest, 27 days after all the series had begun to receive nutrient solutions.

Microchemical tests for starch were made at frequent intervals during this period. The results shown in table 3 indicate clearly that the onset of the

TABLE 3
Results of microchemical starch tests

SERIES	MARCH 17	MARCH 24	MARCH 29
1	xxxxx*	xxxx	xxx
1†	xx	0	0
2	xxx	xxxx	xx
3	xx	xxx	x to xx
4	x	0	0
5	xx	xx to xxx	xxx
6	x	x	x to xx
7	x	x	x to xx

* x indicates the degree of starch concentration found by microchemical tests.

† Shaded side of greenhouse.

initial symptoms of potassium deficiency was associated with an accumulation of starch. This accumulation occurred, however, only with plants situated in the half of the greenhouse that received the most light. The weather during this period of the experiment was cloudy, and, consequently, plants in the part of the greenhouse receiving the least light were growing virtually under winter light conditions. As shown in a previous paper (15), carbohydrates do not accumulate in potassium-deficient tomato plants grown under such conditions; hence, no starch accumulation was found in such plants. All the plants for the first harvest were taken from the light side of the greenhouse. The plants on the darker side were then shifted to the light side and used for the second harvest. Since, as will be shown, the end result of potassium deficiency is to reduce carbohydrates to a low level, the plants thus shifted undoubtedly presented the same external and internal deficiency symptoms at the time of the second harvest as would the plants grown originally on the lighter side of the greenhouse.

As shown in table 3, the starch accumulation in series 1, 2, and 3 gradually decreased. Series 4 had, for some unknown cause, but little starch at any time. Series 5, 6, and 7 gradually increased in starch content, but at the time of the first harvest, only series 5 had any appreciable amount of starch. When the plants were harvested, the tomatoes of series 1 were just beginning to turn dark green, marking the beginning of the "second" stage of potassium deficiency symptoms.

The growth results at the time of the April 1 harvest indicate that 45 p.p.m. of potassium was the lowest amount of potassium which could be used to grow normal tomato plants under the experimental conditions. From these results, it would seem that a definite internal concentration of potassium must be maintained in the plant, or potassium deficiency will result. In solution culture, comparatively low potassium concentrations (0.5 and 2.0 p.p.m.) have been found sufficient to maintain normal growth (6). Under such conditions the roots are constantly bathed in the nutrient solution and are able to absorb enough potassium from these dilute solutions to maintain normal growth. It is apparent, then, that sand culture conditions differ markedly from those of solution culture insofar as absorption of nutrients by the plant is concerned.

The possible role of potassium as a catalyst in plant metabolism will be discussed more fully in the third paper of this series. It may be stated at this point, however, that the possible catalytic action of potassium is dissimilar to that of such elements as iron, boron, and manganese, which act in very small traces. Thin cross sections of tomato petioles were treated with sodium cobaltinitrite, the excess being washed off. The precipitate of potassium sodium cobaltinitrite was clearly visible under the microscope. The cross sections were then treated with ammonium polysulfide, which precipitated the cobalt as the black cobalt sulfide.

Plants receiving 5 p.p.m. of potassium had a surprisingly high concentration of this element, yet the deficiency symptoms came almost as soon and were as severe as those in plants which received no potassium and which on examination showed a very low potassium content. The series receiving 2.5, 11, and 22 p.p.m. of potassium also had comparatively high concentrations of potassium. The complete series, 45 and 175 p.p.m., had higher concentrations of potassium than the deficient series, as was to be expected, but these concentrations were not so high in proportion as might be expected from the greater amounts of potassium present in the nutrient solutions of these series.

At the time of the first harvest, April 1, there were but small differences in height between the potassium deficient plants and those which received 45 and 175 p.p.m. of potassium, but the plants receiving the complete potassium treatment had in every case a much greater volume of growth. The leaves were larger and more numerous in series 7 than in series 1, 2, 3, 4, and 5. The stems in series 7 were much thicker than those of the various potassium deficient series. In striking contrast, however, were the plants in series 6,

which received 45 p.p.m. of potassium. Here the plants were not only greater in height, but also had a greater volume of growth than the plants in series 7.

After the April 1 harvest, the plants were allowed to grow until May 24, 1937. During this period the starch accumulation in the various potassium deficient series gradually disappeared. At the same time brown-red spots began to appear on the lower leaves. The edges of these leaves curled upward, and the leaves began to die progressively up the stem. At the final harvest, almost all the leaves on the series receiving 0 p.p.m. of potassium were dead. Approximately one-half to two-thirds of the leaves on plants receiving 2.5, 5, and 11 p.p.m. of potassium had died at this time, and approximately one-third of the leaves on the 22 p.p.m. series were dead.

The minus potassium plants began to set fruit earlier than the other series, possibly because of the high carbohydrate-nitrogen ration then present in these plants. This condition has been postulated by Kraus and Kraybill (7) as necessary for the formation of fruit in the tomato. Relatively few fruits, however, were formed in the course of the experiment. As will be shown, the carbohydrate content of potassium deficient tomato plants falls to such low levels that the carbohydrate-nitrogen ratio is eventually very unfavorable for fruit formation. Many of the fruits in the minus potassium series dropped off, apparently as a result of the premature formation of an abscission layer.

The plants in series 2, 3, 4, and 5 had a relatively large number of fruits, formed at the expense of the potassium and carbohydrates in the vegetative parts of the plants. Indeed the fruits in series 5 were as large as those in series 6. The plants receiving 45 p.p.m. of potassium at the May 24 harvest still exhibited optimum growth. Fruit was set far more abundantly than in any other series. On the other hand, the plants in series 7 had set very little fruit, in many cases much less than the potassium deficient series. These plants strongly resembled the high nitrogen, strongly vegetative, and sterile plants described by Nightingale, Robbins and Schermerhorn (11). The stems were very thick, and the volume of leafy material was very abundant. Apparently almost all the organic materials synthesized by these plants had been used for vegetative development. It would seem, then, that the carbohydrate-nitrogen ratio of these plants was very low. Chemical analysis of the vegetative parts of the plants of series 7 did not show this, since the enormous drain of carbohydrates from the vegetative parts of the tomato to the fruits did not take place in these plants.

The growth of the plants supplied with varying amounts of potassium is shown in table 4A, and figure 1. The green weights of the stems and fruits and the diameters of the stems and fruits are shown in table 4B and figure 1. These results show clearly the influence of small increments of potassium, up to 45 p.p.m., on the volume and quality of growth. After 27 days the plants in the various series showed only slight differences in height, with the exception of those in series 6, which were noticeably taller than the others.

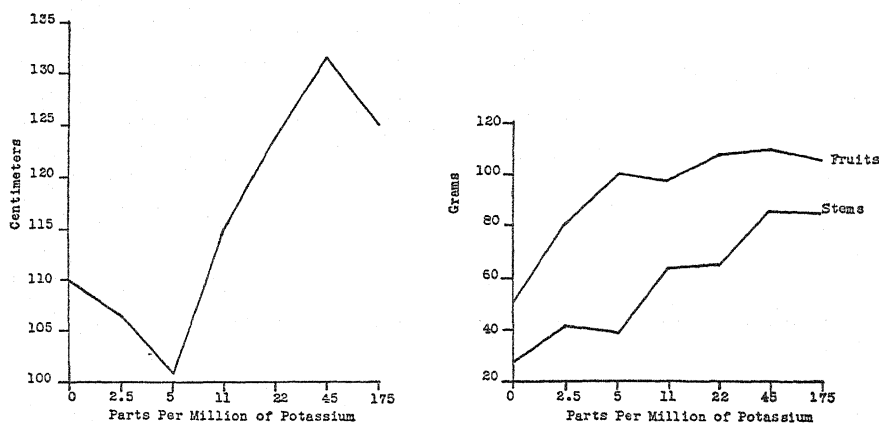


FIG. 1. TOTAL HEIGHT (LEFT) AND GREEN WEIGHT OF STEMS AND FRUITS (RIGHT) OF TOMATO PLANTS GROWN WITH VARYING POTASSIUM CONCENTRATIONS—SECOND HARVEST

TABLE 4A

Height of tomato plants grown with varying amounts of potassium

SERIES	HEIGHT OF PLANTS							TOTAL GROWTH ON NUTRIENT TREATMENT
	1 day	4 days	12 days	14 days	20 days	27 days	82 days	
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
1	22	25	30	33.5*	36.5	46	130	108
2	21	25	30.5	33.5*	39.5	47.5	127.5	106.5
3	19	22	27.5	28.5	33.5*	43	120	101
4	19	22.5	27.5	29.5	35.5*	46	135	115
5	18.5	21.5	29	30.5	34	43*	142.5	124
6	18.5	22.5	28.5	31.5	35.5	50	150	131.5
7	17.5	21	27	28.5	31.5	42	142.5	125

* First deficiency symptoms noted.

TABLE 4B

Average weight and diameter of stems and fruits of tomato plants grown with varying amounts of potassium

SERIES	WEIGHT OF STEMS		WEIGHT OF FRUITS	DIAMETER OF STEMS	DIAMETER OF FRUIT
	April 1	May 24	May 24	May 24	May 24
	gm.	gm.	gm.	cm.	cm.
1	16.4	28.2	52	1.3	5.1
2	21.7	41.1	80	1.6	5.4
3	20.0	38.9	100	...	5.8
4	22.6	63.6	98	2.0	5.8
5	20.0	64.5	112	2.0	6.8
6	33.4	85.7	113	2.2	6.6
7	25.1	85.3	109	2.4	...

After 91 days, the heights of the potassium deficient plants were much less than those receiving 45 p.p.m. From table 4A it may be seen that with the exception of series 1 and 3, increases in height of stems were shown with increasing additions of potassium up to 45 p.p.m. Further addition to 175 p.p.m. of potassium showed a slightly depressing effect.

The effects of potassium deficiency were even more marked on stem and fruit weights and stem and fruit diameters. The minus potassium plants in all cases showed the lowest values for the aforementioned data. An increase in potassium up to 22 p.p.m., with one exception, was associated with increased fruit weights; and up to 45 p.p.m., except for series 3, with increased stem weights. Plants receiving 22 p.p.m. exhibited smaller diameter and stem weight, while the fruits were about equal in size and weight to those receiving 45 p.p.m. The increase in size of fruit in this particular case took place at the expense of the vegetative organs of the plants receiving 22 p.p.m. Though the weights and diameters of the fruit in the series receiving 2.5, 5, and 11 p.p.m. of potassium were in general less than those in the more completely fertilized series, there was not so much difference as in the corresponding stem weights and diameters. The drain on potassium deficient plants is much greater proportionately than the drain on normal plants. These results would seem to show, then, that the tendency in potassium deficient plants is to form fruits approaching normal size and weight at the expense of the vegetative portions of the plants. As will be shown, the potassium and carbohydrate content of the fruits of potassium deficient plants is surprisingly high; facts which verify the preceding statements. The explanation for such a process is very obscure, and cannot be supplied from the data of this experiment.

The shape of the curves of total linear growth and of stem weights is of interest. The curves, in general, drop from 45 to 0 p.p.m. of potassium, and also, less sharply, from 45 to 175 p.p.m. Gassner and Goeze (4) have found similar results for carbon dioxide assimilation, transpiration, and chlorophyll content. Their maximum point was at a potassium concentration in the neighborhood of 40 p.p.m. Wall (15) obtained similar results for total carbohydrate content in tomatoes in a previous investigation.

Nitrogen metabolism

Since most of the analytical data on nitrogen metabolism were obtained from fresh material, the results are presented on a green-weight basis.³ The ratio of soluble organic nitrogen to protein nitrogen was calculated in each case. This ratio is a convenient method of expressing the relative proportions of the two nitrogenous fractions; i.e., when soluble organic nitrogen was high, protein nitrogen was proportionately low, and when soluble organic

³ The complete data for the nitrogenous fractions may be found in the author's original doctoral thesis, "The Role of Potassium in Plants," on file in the Rutgers University Library, New Brunswick, N. J.

nitrogen was low, protein was proportionately high. The leaves were high in protein nitrogen and comparatively low in soluble organic nitrogenous fractions. The reverse held true for the stem fractions. The fruits, however, were very low in absolute quantities of protein and soluble organic nitrogen.

A decrease in the potassium concentration of the nutrient solution was associated with an increase in the plant of all nitrogenous fractions except nitrate nitrogen. Nitrate nitrogen in the very deficient potassium series (0, 2.5, and 5 p.p.m.) tended to be lower in concentration than that in the series receiving more potassium. The effect of potassium concentration on the nitrogenous fractions in the plant was very striking. At the first harvest, the plants receiving 0, 2.5, and 5 p.p.m. of potassium showed an increase in total nitrogen and soluble organic nitrogenous fractions over those receiving 11 and 22 p.p.m., which were similar to plants receiving 45 and 175 p.p.m. At the second harvest, 53 days later, the plants receiving 11 and 22 p.p.m. of potassium showed a higher total nitrogen and soluble organic nitrogen content than those receiving 45 and 175 p.p.m. At no time was there any significant difference in nitrogen fractions between the two high potassium series.

The upper portions of the plant, as mentioned previously, presented the same general trends as the lower portions of the plant. The curves for nitrogenous fractions, drawn from data for the upper leaves and upper stems (fig. 2), are therefore representative of the general trend of the nitrogenous fractions in the vegetative organs of the plant. The nitrogenous fractions in the fruit fluctuated, and no definite trends could be shown.

The results in figure 2 show very clearly that increasing increments of potassium in the nutrient solution up to 45 p.p.m. were associated with a decrease in the concentration of nitrogenous fractions in the tissue and that beyond this point additional amounts of potassium had but little effect. The main effect of potassium deficiency was to increase the concentration of soluble organic nitrogenous fractions such as ammonia, amide, and amino nitrogen. Total nitrogen and protein nitrogen were also higher, on a percentage of green weight basis, in the series showing potassium deficiency. On a quality basis, the protein content fluctuated, being in some cases higher and in others lower than that of the high potassium series.

The opinion has been expressed by Schmalfuss (12) and Gregory (5) that the high protein and total nitrogen contents of potassium deficient plants are in reality due to the small size of such plants as compared to completely fertilized plants. This tends to cause a comparatively low protein content to appear high on a concentration basis.

The role of potassium in nitrogen metabolism of plants will be more completely presented in the following paper of this series. The evidence presented by the data of this experiment is largely indirect. Increases in potassium up to 45 p.p.m. will for some time keep the nitrogen concentrations close to that of completely fertilized plants. In time, however, concentrations as

high as 22 p.p.m. of potassium were not sufficient to prevent the typical increase in soluble organic nitrogen associated with potassium deficiency. The increase in soluble organic nitrogen is usually inversely proportional to the

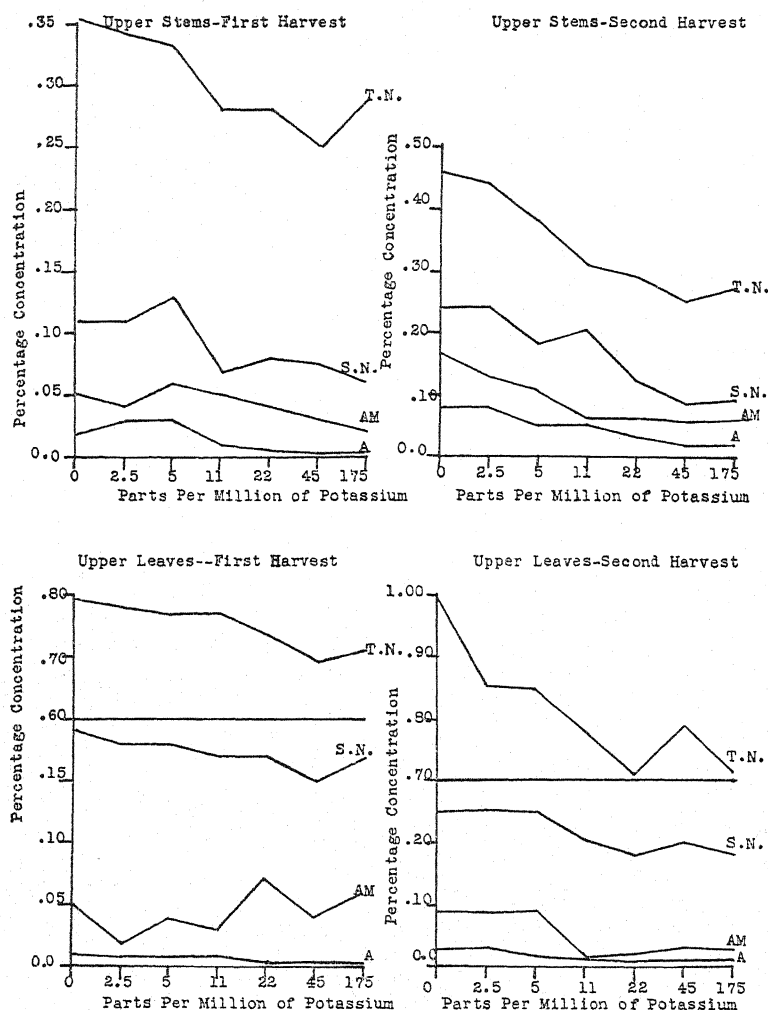


FIG. 2. NITROGENOUS FRACTIONS OF UPPER LEAVES AND UPPER STEMS OF TOMATOES GROWN WITH VARYING POTASSIUM CONCENTRATIONS

T.N. = Total nitrogen; S.N. = soluble organic nitrogen; A = ammonia nitrogen;
AM. = amino nitrogen

potassium content, and if increase in soluble organic nitrogen content is to be taken as one of the typical internal symptoms of potassium deficiency, the lower values for this fraction found with increasing additions of potassium up to 45 p.p.m., should tend to prove that potassium does play a definite

role in nitrogen metabolism. This increase in soluble organic nitrogen may be due in the early stages to interference with the synthetic processes and may be accompanied later by rapid proteolysis. Moreover, carbohydrate accumulation was noted in the potassium deficient plants at the April 1 harvest. This accumulation of carbohydrates was also inversely proportional to potassium content. This would seem to be direct proof that synthetic processes are involved, since carbohydrates will not accumulate unless some stage of nitrogen metabolism has been checked.

For several reasons it is difficult to say, from the results of this experiment, precisely what stage of the nitrogen metabolism is affected by potassium deficiency. In the first place, the fractionation of the vegetative parts of tomato into upper and lower portions of the stems and leaves may lead to error, since the tomato plants vary greatly in composition from top to bottom, and furthermore in potassium deficient plants the tops are not affected to the same extent as the middle and bottom portions. Secondly, the processes of synthesis and proteolysis of proteins are continually occurring, with the result that it is difficult to determine whether an accumulation of soluble organic nitrogen in potassium deficient plants is due to a primary decrease in synthetic processes or of secondary origin, due to the abnormally rapid hydrolysis of proteins. Both processes may take place at the same time, thus further obscuring the issue. In the early stages of potassium deficiency, where ample carbohydrates are present, abnormally rapid proteolysis probably will not take place. Since at this stage the plants receiving 0, 2.5, and 5 p.p.m. of potassium were showing definite increases in ammonia and soluble organic nitrogen concentrations in conjunction with an accumulation of carbohydrates, it may be concluded that some stage in the protein synthetic process was affected. The very great increases in soluble organic nitrogenous fractions at the second harvest are probably partly due to this check in synthetic processes, and partly due to rapid hydrolysis of proteins, for at this stage the carbohydrate content of potassium deficient tomatoes was very low.

Carbohydrate metabolism

Some of the results for the various carbohydrate fractions, on a dry-weight basis, are shown in figure 3.⁴ The curves are fairly representative of the trend of results in all the plant fractions except the fruits. Hemicellulose was determined, but is not presented, since in all cases this analytical fraction was very low. Fructose was found in large quantities in the fruits, but only extremely small concentrations were found in the vegetative organs. Starch and reducing sugars were abundant in all vegetative parts of the plant, but sucrose was low in the leaves and somewhat higher in concentration in the stems. The stem of the tomato evidently acts as a storage organ, since carbohydrate concentrations were much higher than in the leaves.

⁴ For complete data refer to original thesis.

The effect of increasing amounts of potassium on the carbohydrate concentrations in the plants was very noticeable. At the first harvest, the carbohydrate and dry matter content were in inverse proportion to the potassium

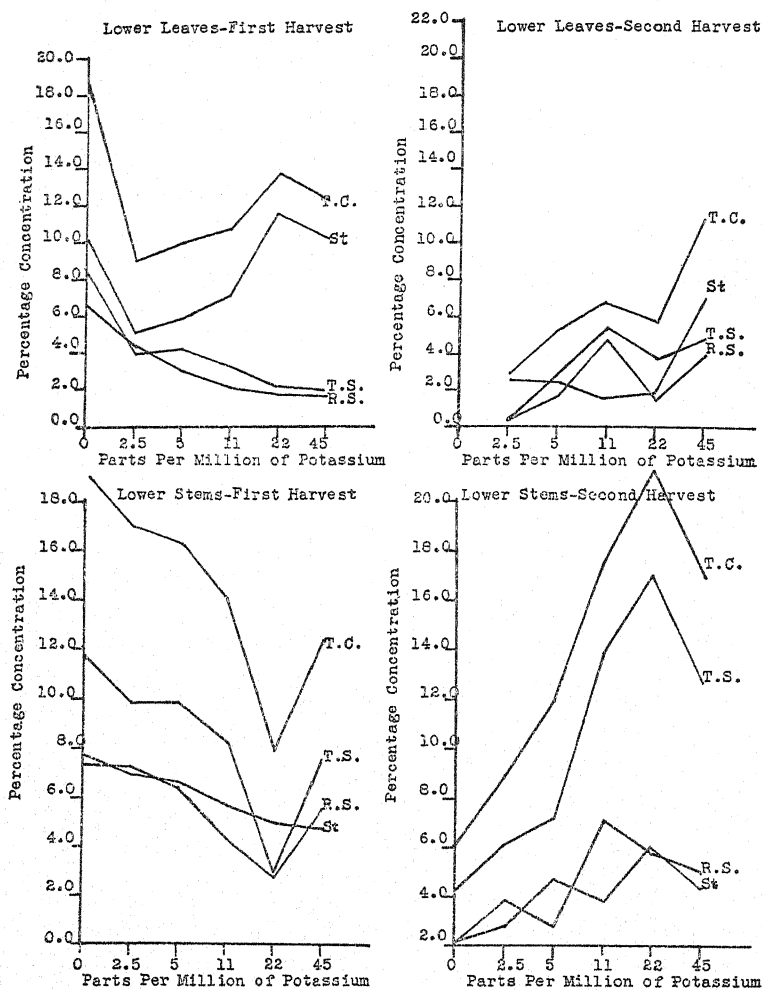


FIG. 3. CARBOHYDRATE FRACTIONS OF LOWER LEAVES AND LOWER STEMS OF TOMATOES GROWN WITH VARYING POTASSIUM CONCENTRATIONS

St = Starch; R.S. = reducing sugar; T.S. = total sugar; T.C. = total carbohydrate

concentrations supplied. The plants receiving very low potassium, 0, 2.5, and 5 p.p.m., had a much greater total sugar and a somewhat greater starch concentration than plants receiving 11, 22, 45, and 175 p.p.m. The plants receiving 11 and 22 p.p.m. of potassium were higher in carbohydrate content than those receiving 45 p.p.m. The plants receiving 175 p.p.m. had a rather

high carbohydrate content at this time, but the carbohydrate results for this series must be discounted for both harvests, since the plants set very little fruit in comparison to the other series, and therefore, all the carbohydrates which would have been translocated to the fruits remained behind in the vegetative organs. This caused series 7 apparently to have a very high carbohydrate content.

At the second harvest, the results were reversed. Here the carbohydrate content tended to increase as the potassium concentration was increased up to 45 p.p.m. The total carbohydrate content of plants receiving 0, 2.5, and 5 p.p.m. of potassium was especially low, whereas that of plants receiving 11 and 22 p.p.m. fluctuated, being higher in some parts of the plant and lower in others than that of plants receiving 45 p.p.m. The lower portions of the plants receiving 11 and 22 p.p.m. of potassium were much lower in carbohydrate content than the corresponding portions of plants which received 45 p.p.m. If the experiment had continued longer, series 4 and 5 also would undoubtedly have shown a low carbohydrate content, since these plants were showing only moderately severe symptoms of potassium deficiency at the time of harvest. The upper portions of these plants, however, had a carbohydrate content as high as or higher than that of the plants of series 5. This may have been due to the translocation of potassium from the lower to the upper regions of the plants of series 4 and 5.

The fruits which were analyzed at the second harvest did not show the same trend of results. Here the fruits of plants receiving 0, 2.5, and 5 p.p.m. of potassium had a considerably higher carbohydrate concentration than the fruits of the other series. The series receiving 11, 22, 45, and 175 p.p.m. of potassium had approximately the same carbohydrate concentrations.

The accumulation of carbohydrates in potassium-deficient tomatoes is an important but temporary phase in the metabolism of such plants and is undoubtedly due to interference with some stage in the synthesis of proteins. The fall in carbohydrate content in the later stages of potassium deficiency is more typical of potassium deficiency.

The drain of the fruits on the carbohydrate reserves of low potassium plants serves to accentuate the effect of insufficient potassium on the carbohydrate metabolism, but it apparently is not the cause of the low carbohydrate concentrations in such plants. As in the case of the nitrogen metabolism, increasing increments of potassium up to 45 p.p.m. bring the carbohydrate metabolism closer to the optimum. Such a result is perhaps to be expected, for, as will be shown in another paper, the effects of potassium on the carbohydrate metabolism are closely interrelated with the effects of potassium on the nitrogen metabolism of the tomato plant.

Mineral metabolism

Since all the mineral analyses were conducted on dried tissue, the data on mineral metabolism are reported on a dry-weight basis for the sake of con-

venience. Analyses for sodium were conducted but are not recorded, since few significant differences were noted.

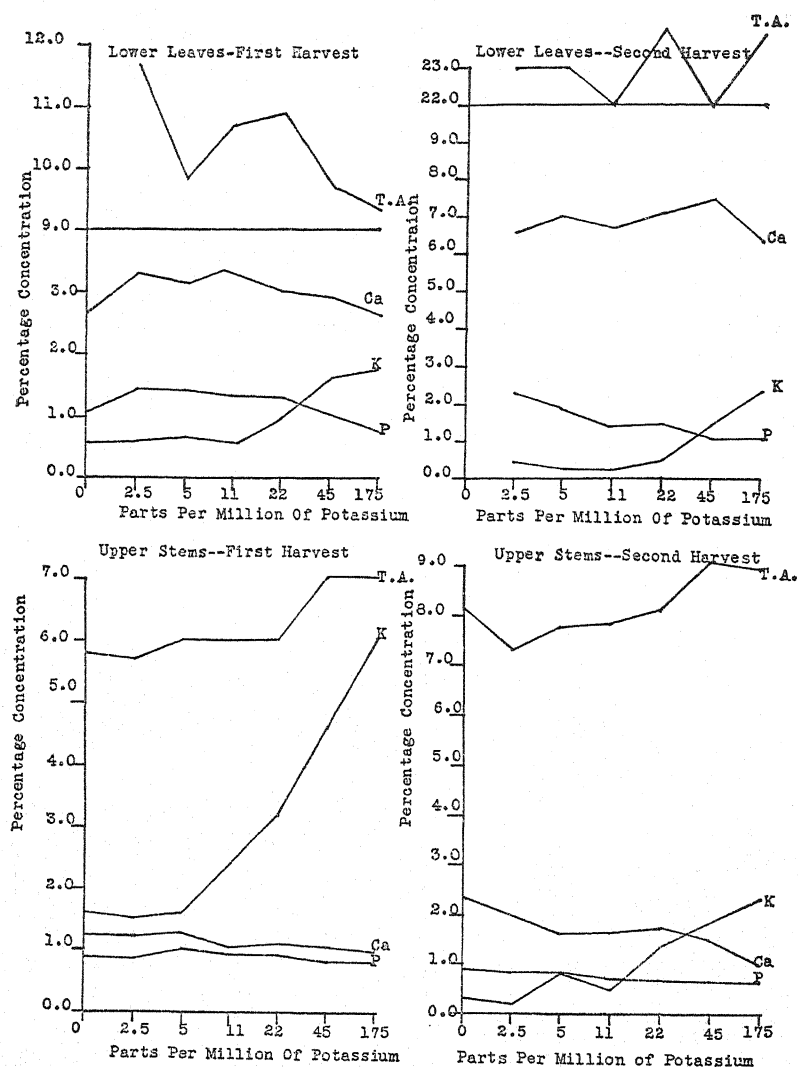


FIG. 4. MINERAL FRACTIONS OF THE LOWER LEAVES AND UPPER STEMS OF TOMATO PLANTS GROWN WITH VARYING POTASSIUM CONCENTRATIONS

T.A. = Total ash

In general, the leaves of the tomato plants were higher in calcium, magnesium, and phosphorus but lower in potassium than the stems. The fruits were very low in all minerals except potassium, which was proportionately higher. Calcium and magnesium were much higher in the lower leaves

than in the upper leaves. Such great differences however, did not exist between the upper and lower stems. Potassium on the other hand, was generally greater in concentration in the upper leaves and stems than in the corresponding lower fractions. Total ash was always higher in the leaves than in the stems. The lower leaves were much higher in total ash than the upper leaves, whereas the upper stems were somewhat higher in ash content than the lower stems.

The varying potassium concentrations had no effect on the general distribution of minerals previously mentioned, but did affect the concentrations of the individual ions. The potassium concentrations, as might be expected, increased with increasing addition of potassium, but very little difference was shown by plants receiving 0, 2.5, and 5 p.p.m. of potassium. The concentration began to rise slightly when 11 p.p.m. of potassium was supplied, and rose more noticeably when 22 p.p.m. was supplied. The increments of 45 p.p.m. and 175 p.p.m. gave rise to marked increments in internal potassium content. The failure of the very small increments of potassium supplied to produce corresponding increments in internal potassium concentration may be explained in two ways: first, the small increments in potassium in the external solution cause a corresponding increment in the volume of growth, which keeps the concentrations of potassium approximately the same; second, almost all the available potassium in the very low potassium plants is translocated in approximately the same concentrations to the fruits, the amounts remaining in the vegetative portions of the plants being too small for any definite differences to be shown.

As mentioned previously, the fruits of all the series have a very high potassium content. In proportion to the concentrations of potassium in the vegetative organs, however, the concentrations in the fruits of the deficient series (0, 2.5, 5, 11, and 22 p.p.m.) are relatively higher than the concentrations in fruits of plants supplied with complete potassium (45 and 175 p.p.m.). This high drain on potassium reserves by the fruits, together with the fact that the constant absorption of potassium in the deficient levels cannot keep pace with the dilution effect of the increased volume of growth caused by such absorption, is responsible for the deficiency symptoms which appear in plants constantly supplied with 11 and 22 p.p.m. of potassium. It may also be postulated that potassium deficiency will tend to check the ability of the plant to absorb ions, and thus will increasingly reduce the absorption of potassium by the roots. Very probably, however, the ion-absorbing capacity of the roots of potassium deficient plants which are not actually dying is not diminished, since the intake of other ions is actually increased in potassium deficiency.

Phosphate content in general was decreased by the addition of increments of potassium in the external solution. Calcium showed the same trend in the stems but fluctuated somewhat in the leaves. Magnesium was not greatly affected by variations in potassium content, although trends similar to those

for phosphate and calcium were found in the lower leaves at the second harvest. The increase in absorption of other ions in the absence of potassium is believed to be due to the high mobility of potassium, which when present will tend to repress the absorption of other ions (9).

The higher ash content found with increasing potassium is undoubtedly due to the greater absorption of potassium and accompanying anions. The increase in absorption of other ions by potassium deficient plants is evidently not great enough to counterbalance the effect on ash weight by the increased absorption of potassium.

The data for the lower leaves and the upper stems are shown graphically in figure 4.

SUMMARY

Rutgers tomato plants were grown at 0, 2.5, 5, 11, 22, 45, and 175 p.p.m. of potassium respectively. All the various series except those supplied with 45 and 175 p.p.m. showed some degree of potassium deficiency. The severity of the deficiency symptoms was roughly proportional to the amount of potassium supplied. The growth data curves showed a rapid increase with increments in potassium supply until 45 p.p.m. of potassium was reached. Above this point the growth curves slowly declined.

Two types of potassium deficiency symptoms were noted. The first stage appeared early in the course of the experiment, and was marked by a stunted, hard, yellow plant. In the second stage, the plants began to grow, turned green, and became soft. At the same time the lower leaves began to die progressively up the stem. The first stage of potassium deficiency was associated with a high carbohydrate content in the low potassium plants. In the second stage, the carbohydrate content of these plants greatly diminished. The potassium deficient plants were characterized by a much higher soluble organic nitrogen and total nitrogen content than the plants receiving the complete solutions. The initial carbohydrate accumulation and final decrease, and the high soluble organic nitrogen content were most noticeable in the completely potassium deficient plants. As more potassium was supplied, the plants in the various series approached closer and closer to normal plants in carbohydrate and nitrogen concentrations.

The potassium content of the plants slowly increased with increasing potassium up to 22 p.p.m. At this point increments of 45 p.p.m. and 175 p.p.m. of potassium caused sharp increases in the potassium content. Calcium, magnesium, and phosphates were generally higher in concentration in the low potassium plants.

REFERENCES

- (1) BECKENBACH, J. R., WADLEIGH, C. H., AND SHIVE, J. W. 1936 Nutrition studies with corn: I. A statistical interpretation of the nutrient ion effect upon growth in artificial culture. *Soil Sci.* 41: 469-488.

- (2) CLARK, H. E. 1936 Effect of ammonium and nitrate nitrogen on the composition of the tomato plants. *Plant Physiol.* 11: 5-24.
- (3) DAVIDSON, O. W., AND SHIVE, J. W. 1935 Determination of the nitrogenous fractions in the vegetative tissue of the peach. *Plant Physiol.* 10: 73-92.
- (4) GASSNER, G., UND GOEZE, G. 1934 Assimilationsverhalten, Chlorophyllgehalt, und Transpirationsgrosse von Getreideblättern mit besonderer Berücksichtigung der Kalium und Stickstoffernährung. *Ztsch. Bot.* 27: 257-340.
- (5) GREGORY, F. G. 1937 Mineral nutrition of plants. *Ann. Rev. Biochem.* 6: 557-578.
- (6) JOHNSTON, E. S., AND HOAGLAND, D. R. 1929 Minimum potassium level required by tomato plants grown in water cultures. *Soil Sci.* 27: 89-109.
- (7) KRAUS, E. J., AND KRAYBILL, H. R. 1918 Vegetation and reproduction with special reference to the tomato. *Oreg. Agr. Exp. Sta. Bul.* 149.
- (8) LINK, K. P. 1923 Effects of the method of desiccation on the carbohydrates of the plant tissue. *Jour. Amer. Chem. Soc.* 45: 439-447.
- (9) LUNDEGÅRDH, H. 1934 Mineral nutrition of plants. *Ann. Rev. Biochem.* 3: 485-498.
- (10) NIGHTINGALE, G. T., ROBBINS, W. R., AND SCHERMERHORN, L. G. 1927 Freezing as a method of preserving plant tissue for the determination of nitrogenous fractions. *N. J. Agr. Exp. Sta. Bul.* 448.
- (11) NIGHTINGALE, G. T., ROBBINS, W. R., AND SCHERMERHORN, L. G. 1928 The growth status of the tomato as correlated with organic nitrogen and carbohydrates in the roots, stems, and leaves. *N. J. Agr. Exp. Sta. Bul.* 461.
- (12) SCHMALFUSS, K. 1932 Untersuchungen über den Eiweissstoffwechsel von Kalimangelpflanzen. *Phytopath. Ztschr.* 5: 207-249.
- (13) VAN DER PLANK, J. E. 1936 The estimation of sugars in the mangold. *Biochem. Jour.* 30: 457-469.
- (14) VICKERY, H. B., PUCHER, G. W., AND CLARK, H. E. 1935 The determination of glutamine in the presence of asparagine. *Biochem. Jour.* 29: 2710-2720.
- (15) WALL, M. E. 1939 The role of potassium in plants: I. The effect of varying amounts of potassium on nitrogenous, carbohydrate, and mineral metabolism in the tomato plant. *Soil Sci.* 47: 143-161.
- (16) WALL, M. E. The micro determination of some constituents of plant ash. *Plant Physiol.* (in press).

IRON-MANGANESE CONCRETIONS IN DAYTON SOILS

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In connection with a detailed field and laboratory investigation of Dayton and associated soils, many data were obtained on concretions, which are a prominent feature of the Dayton soils. The results of this phase of the study are reported in this paper.

The presence of a large quantity of concretions was recognized as a characteristic property of the Dayton soil in the first systematic description of that series appearing in the Soil Survey Report of Yamhill County, Oregon, in 1917 (4). It was mentioned that, in this soil (locally known as "white land") of the poorly drained open prairie, "small brownish pellets of iron concretions are usually abundant in both the soil and the subsoil."

Recently it was found (5) in a morphological study of the Dayton soil that "the occurrence, in great numbers, of small well-rounded dark brown or black ferric concretions (ortstein) is a typical feature of this A_2 horizon. The concretions range from those hardly visible to the naked eye to those about one-half inch in diameter and generally are firm enough to resist crushing, even by strong pressure. They appear in the soil from the top of the A_2 horizon and are especially numerous in the A_2 lower subhorizon." The Dayton soils were classified as an intrazonal glei-meadow podzol the morphology of which is "strikingly similar to that of the so-called 'solodi'—an intrazonal soil found in the grassland belt (prairie and steppe) where it similarly occupies the depressed and poorly drained areas." These soils are characterized by a very light gray, almost white, A_2 horizon, which ranges in thickness from about 8 inches to more than 2 feet. Material of this horizon usually has the texture of a silt loam, a rather crumbly consistence, and a weakly developed lumpy structure and is acid. The lower boundary of the A_2 is rather abrupt. Below this horizon is a very dark gray, very compact, clayey B, which averages 12 or 14 inches in thickness. Very sticky and almost impervious when moist, this material breaks into large, irregular, roughly prismatic clods when dried. The lower boundary of the B horizon is usually indistinct. It grades without a sharp line of demarcation into the lighter colored yellowish gray C, in which the percentage of clay gradually decreases with depth.

These soils develop on poorly drained terraces of the Willamette River Valley in Oregon. The impervious character of the B horizon causes a water-logging of the upper horizons during the wet seasons, winter and spring.

Usually such a condition exists during a continuous period of about 6 months annually.

DISTRIBUTION OF CONCRETIONS

The concretions are present throughout the A and B horizons of the Dayton soils; in the A₁ and B, however, their number is not very large. They increase both in number and in size in the A₂ and especially in the central and lower parts of this horizon, then sharply decrease throughout the B both in number and in size. In the upper C the firm concretions are rather rare.

The approximate amounts of concretions of various sizes found in different horizons of one profile of Dayton silt loam are presented in table 1. The results are expressed in per cent by weight of the whole soil. For this determination, 200 gm. of air-dry soil was carefully rubbed in water until all non-cemented aggregates of the soil were dispersed. The fine material was re-

TABLE 1
Distribution of iron-manganese concretions in a profile of Dayton silt loam

DIAMETER OF CONCRETIONS	A ₁ 0-2 INCHES	A ₂ 2-6 INCHES	A ₂ 6-10 INCHES	A ₂ 10-14 INCHES	B 14-18 INCHES	B 18-22 INCHES	B-C 30-34 INCHES	C 42-50 INCHES
mm.	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
>2	1.7	3.0	8.0	6.0	None	None	None	None
2.00-1.00	0.75	1.6	2.5	1.5	1.1	1.0	.1	None
1.00-0.5	0.75	1.6	0.5	1.8	1.1	0.9	.5	.4
0.5-0.25	0.3	0.4	0.2	0.6	0.6	0.4	.2	.3
0.25-0.1	0.1	0.1	0.1	0.4	0.4	0.2	None	.2
0.1-0.05	0.1	0.1	0.1	0.2	<0.1	<0.1	None	None
Total.....	3.70	6.8	11.4	10.5	3.2	2.5	.8	.9

moved by decantation, and the coarse material was washed, dried, and separated into the various sized fractions by sieving. A microscopic examination of these fractions shows that the fine gravel fraction (2-1 mm.) consists entirely of concretions and that about 70 per cent of the coarse sand (1-0.5 mm.), about 50 per cent of medium sand (0.5-0.25 mm.), and about 30 per cent of the fine sand (0.25-0.1 mm.) are also represented by the concretions. The quantity of concretions below 0.1 mm. size is uncertain.

The quantities of concretions in different profiles vary to some extent, but a relative increase in percentage in the lower part of the A₂ is a common feature of all 28 profiles which were examined in the field. The quantity of concretions in the A₂ horizon of another profile was found to be 5 per cent, whereas in the B horizon of the same profile it was 2.6 per cent. The zone of concentration of concretions is usually just above the surface of the B horizon, although in many instances the concretions in the middle part of the A₂ are more numerous than in the lower part of this horizon.

In the detailed study of the one Dayton profile it was noted that of the con-

cretions greater than 2 mm. the largest ones in the A_1 horizon are about 5 mm. in diameter; most of them are 2-3 mm. In the upper A_2 , the largest are about 7 mm. and most of them are 2-3 mm., but a greater number than in A_1 are larger than 3 mm. In the central and lower A_2 , more than half of the concretions greater than 2 mm. in diameter are over 5 mm. The largest ones are from 10-15 mm. This general distribution of concretions of the various sizes has been observed in several profiles of the Dayton soil.

Concretions larger than 2-3 mm. in diameter apparently do not form below the A_2 , and even those about 2 mm. in diameter are few. Most of the concretions in the central part of the B horizon range between 0.5 and about 1.5 mm., whereas in the lower part of B and in the upper part of C very few concretions are larger than 1 mm. in diameter.

The firm concretions, in general, are rather rare in the C horizon. The upper part of the C of most profiles, however, is marked by minute dark brown or black specks. An examination of these under a magnifying glass indicates that they are formed by segregations of presumably the same material by which the firm concretions are cemented. It is possible that such soft segregations differ from concretions mainly in the lack of cementation.

A diameter of about 0.1 to 0.05 mm. probably represents the lower limit of the size of concretions. Microscopic examinations of the finer soil separates do not show the presence of concretions of diameter less than 0.05 mm.

The concretions in the A_2 horizon range in diameter from less than 0.1 mm. to more than 15 mm. Usually they are somewhat irregular in shape, although they tend to be rounded. The larger ones are more irregular in shape; some of them apparently are clusters of several smaller ones cemented together. The color of the concretions ranges from light brown to black, the large ones usually with a metallic blue luster in the kernel and a rusty or ochre-yellow shell around the black nucleus. Most of the concretions are very well cemented and difficult to crush, whereas some are less firm and crush even under moderate pressure.

PREVIOUS INVESTIGATIONS

Different kinds of concretions have been described in widely different types of soils. Wheeting (17) has studied the concretions in the shot soils of Washington. Roberts (8) has found a large number of concretions in many soils of Puerto Rico. The Tifton soil series of southeastern United States is characterized by the presence of a large number of concretions throughout the solum.

Concretions similar to those of the Dayton soil have been described by several authors. Some investigators regard them as a peculiar form of ortstein. Glinka (2) states that "ortstein of the clayey soils appears in the form of disconnected concretions which are rounded in shape and range in diameter from 1 or 2 millimeters to more than 1 centimeter." Tumin (15) points out that the true ortstein develops in sandy soils only, whereas

the iron and manganese concretions form in heavy soils. Neither ortstein in the podzolic sandy soil nor concretions in the heavier podzolic soils, however, are always present. Both ortstein and concretions develop only in a certain environment. It is noteworthy that ortstein develops at the boundary between the A_2 and B horizons, whereas concretions occur throughout the profile. Tumin concludes that the general facts regarding ortstein and concretions indicate that both form as a result of the reoxidation of certain compounds in the soil.

Tumin reports also on the chemical composition of concretions from different horizons of one profile of a podzol from the Smolensk province in Russia. He finds that concretions in the A_1 horizon contain 18.12 per cent of Mn_3O_4 , those in A_2 only 2.46 per cent, and those in the B horizon 5.06 per cent.

Popov (7) finds a rather common occurrence of ortstein concretions the size of a pea in the A_2 and occasionally in the B and glei horizons of the glei-podzolic soils and of the solodi soils in poorly drained depressions scattered throughout the Voronej steppe. A particularly large number of such dark rusty brown concretions was noted at the boundary between the A_2 or A_3 and the heavy glei horizon. All soils described by Popov are fine-textured.

Glinka (2) has analyzed the ortstein concretions and the soil material free of concretions of the A_2 horizon of a clayey podzol from Hungary. He reports that the concretions contain 14.49 per cent of Fe_2O_3 and 12.93 per cent of MnO , whereas the soil material in which these concretions were found had only 2.88 per cent of iron oxide and no manganese. The percentage of calcium, magnesium, sodium, and potassium is greater in the concretions than in the soil, whereas silica and alumina are higher in the soil than in the concretions. Analyses by Gemmerling (1), quoted by Glinka, suggest the possibility of the presence of free alumina in the concretions.

Zakharov (19), who has analyzed concretions of different sizes, reports that small concretions (1–1.5 mm.) contain 8.39 per cent of Fe_2O_3 and 1.37 per cent of Mn_3O_4 , concretions of medium size (2–3 mm.) 7.78 and 1.82 per cent respectively, and the large ones (>5 mm.) 6.23 and 2.15 per cent. He concludes that with an increase in size of concretions the percentage of iron oxide decreases and the manganese increases. Zakharov finds also a considerable amount of soluble silica in all the concretions.

Joffe (3) gives a detailed account of ortstein formation, accompanied by an exhaustive review of literature on this subject. He states that "another type of ortstein formation is the concretions, usually found in clay podzols, which appear at the bottom of the A_2 and on top of the B horizon. They contain variable amounts of humus, iron, and sometimes manganese. Their humus content is greater than that of the surrounding soil material."

Tsukunaga (14) describes the iron concretions in certain Manchurian soils as spherical or elliptical concretions of concentric shelly structure. The larger concretions, which form mainly in the humus horizon, are dark brown to

nearly black on the surface and dark brown to yellowish brown inside. This description corresponds to that of the nodules formed in many bog and peaty soils. Tsukunaga points out that some iron-fixing bacteria and the presence of tannins might have played some part in the formation of these concretions.

More recently Winters (18) has made a rather detailed study of concretions in podzolic soils in Illinois. He finds that, in general, concretions are most abundant in the surface horizons of poorly drained, light-colored soils. The concretions are more or less spherical, reddish brown to nearly black, and range in diameter from less than 0.05 mm. to more than 10 mm. They were found to be much higher in manganese and iron than the soil in which they occurred. Segregation of manganese is especially noted. Some concretions have more than 50 times as much manganese as the corresponding soil. Winters reports from 3.6 to 11.5 per cent of Mn_2O_3 and from 14.0 to 24.3 per cent of Fe_2O_3 in concretions, whereas the corresponding soil contained only from 0.06 to 0.16 per cent of manganese and from 1.8 to 3.0 per cent of iron oxides. Larger concretions were found to be higher in manganese than the smaller ones, whereas an increase of iron with the diminishing size of concretion was less consistent. Winters notes also the concentrically layered structure of the concretions and an inclusion, within the concretions, of sand grains and other soil material, which give the impression that the cementing material has been deposited in the pore spaces.

ANALYTICAL DATA

The chemical composition of the profile of Dayton silt loam from which the concretions used in this investigation were separated is presented in table 2.

The chemical composition of several individual concretions of various sizes taken from the central part of the A_2 horizon is given in table 3. The data show clearly the high concentration of iron and manganese in all the concretions analyzed, whereas the percentages of the other constituents do not differ significantly from that of the whole soil. There are, however, significant differences in the percentage of iron and manganese oxides in concretions of different sizes. The large concretions (12 mm.) contain more than four times as much manganese but only about one-half to one-third as much iron as the smaller ones (2 mm.). These data are similar to those reported by Zakharov and by Winters. They indicate a marked segregation of iron and especially of manganese oxides in the concretions, which contain from two to more than four times as much iron oxide and from about 15 to more than 75 times as much manganese oxide as the whole soil material in which these concretions were formed.

It is to be noted from table 2 that the percentage of iron and manganese in the A_1 and A_2 horizons of this soil amounts to about one-half that in the B and C horizons. The A horizon in general and the A_2 in particular represent the zone in which most of the concretions are formed. The data in table 1 indicate that from one-half to about two-thirds of these concretions

are larger than 2 mm. in diameter. Because of the size and hardness of these concretions a considerable part is removed from the material taken for the usual chemical analyses, since only material which passes through the 2-mm. sieve is usually considered as soil. In this way a large part of the concretions, in which much of the iron and manganese is segregated, is removed from the soil before the analysis is made. This is one reason for the relatively low content of iron and manganese in the chemical analysis of the A horizon as compared to the B and C horizons.

TABLE 2
Chemical composition of Dayton silt loam

LABORATORY NUMBER	HORIZON	DEPTH inches	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	K ₂ O	Na ₂ O	MnO	ORGANIC MATTER	IGNITION LOSS	pH
			per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	
C-4226	A ₁	0-2	70.0	12.1	3.5	0.90	0.77	2.04	1.16	.07	5.60	8.24	4.8
C-4228	A ₂	6-10	71.8	13.4	4.3	0.94	0.88	2.18	1.28	.06	1.37	3.97	4.8
C-4229	A ₂	10-14	70.3	14.3	4.8	0.89	1.03	2.00	1.23	.08	0.85	4.13	4.8
C-4231	B	18-22	60.7	18.2	8.4	1.39	1.86	1.35	0.85	.15	0.48	5.96	6.3
C-4233	B-C	26-30	60.9	17.2	8.1	1.98	2.02	1.44	1.08	.13	0.35	5.70	7.2
C-4236	C	42-50	62.7	17.3	7.0	2.09	2.04	1.84	1.25	.13	0.17	4.35	7.0

TABLE 3
Chemical composition of iron-manganese concretions from the A₂ horizon of Dayton silt loam
(Laboratory number of sample C-4228, depth 6-10 inches)

DIAMETER OF CONCRETIONS	SiO ₂	Al ₂ O ₃	Fe ₂ O ₃	CaO	MgO	MnO	IGNITION LOSS
mm.	per cent	per cent	per cent	per cent	per cent	per cent	per cent
12	62.5	12.3	8.4	.56	1.13	4.50	4.93
12	60.8	12.5	9.4	.86	1.03	4.80	5.32
6.0	56.6	12.6	13.3	.57	0.85	3.79	5.80
6.0	62.6	12.6	10.9	.69	0.98	1.91	4.86
2.0*	55.9	12.3	17.8	.69	0.82	1.01	6.25
2.0*	45.8	15.6	25.4	Not determined		1.48	
1*	59.2	15.5	15.2	Not determined		0.38	

* Average for a group.

In order to obtain more definite information on this point, the percentage of manganese was determined on the Dayton soil with all concretions present, and also on samples from which all the concretions were removed by sedimentation of a water suspension of the soils. The data given in table 4 show that the percentage of MnO in the A₁, A₂, and B horizons of the soil containing all the concretions is .09, .24, and .15 respectively, whereas these same horizons free of concretions contain .05, .04, and .03 per cent of MnO. The percentage of MnO in these three horizons with only concretions greater

than 2 mm. in diameter removed is .07, .06, and .15 respectively. These data indicate also that about three-fourths of the total manganese in the A_2 is segregated in concretions larger than 2 mm. in diameter and about one-third of the remainder is in concretions smaller than 2 mm.

The percentage of MnO in concretions of various sizes and from different horizons of the same profile is also presented in table 4. These data confirm the previous conclusion regarding the higher concentration of manganese in the large concretions as compared to the smaller ones. Such a tendency is evident in every horizon taken individually but not when certain sized concretions of the horizon are compared with those of the same size in another

TABLE 4

Percentage of MnO in concretions of different sizes and in the whole soil with and without concretions in different horizons of Dayton silt loam

LABORATORY NUMBER OF SAMPLE	HORIZON	DEPTH	DIAMETER OF CONCRETIONS						WHOLE SOIL INCLUDING ALL CONCRETIONS	SOIL WITHOUT ANY CON- CRETIONS	SOIL WITH CONCRETIONS LARGER THAN 2 MM. REMOVED
			12 mm.	6 mm.	5-4 mm.	2 mm.	2-1 mm.	1-0.5 mm.			
C-4226	A_1	0-2			0.88		.34		.09	.05	.07
C-4227	A_2	2-6			1.90 1.86	1.54	.66				
C-4228	A_2	6-10	4.50 4.80 4.62 3.20	3.78 1.91 1.82 2.58	2.50 1.76	1.48 1.01 1.60		0.38 0.56	.24	.04	.06
C-4231	B	18-22				5.71 5.71		3.33 3.08	.15	.03	.15

horizon. Concretions 2 mm. in diameter from the B horizon contain more MnO than the 12-mm. concretions from the A_2 . Similarly the 1-0.5-mm. concretions from the B contain more manganese than the 6-mm. concretions from the A_2 , and the 2-mm. concretions from the A_2 more than the 4-5-mm. concretions from the A_1 . These data indicate another tendency, namely, increase of concentration of manganese in concretions of the lower horizons in the solum. Since concretions larger than 2 mm. in diameter do not form in the B horizon, only the smaller concretions can be compared in the different horizons. The 2-mm. concretions from the B were found to contain more than three times as much manganese as the concretions of similar size in the A_2 . The 1-0.5-mm. concretions from the B contain more than six

times as much MnO as those from the A_2 . The 4-5-mm. concretions from the A_2 contain more than twice as much MnO as do the similar concretions from the A_1 .

The percentage of Fe_2O_3 in concretions of various sizes and from different horizons is presented in table 5. These data indicate that the percentage of iron oxide in the concretions increases with a decrease in size, to a certain limit. The 2-mm. concretions from the A_2 contain from two to three times as much iron oxide as the 12-mm. ones and about two times as much as the 6-mm. concretions. The content of iron in the very small concretions (<1

TABLE 5

Percentage of Fe_2O_3 in concretions of different sizes and in the whole soil without large concretions in different horizons of Dayton silt loam

LABORATORY NUMBER OF SAMPLE	HORIZON	DEPTH	DIAMETER OF CONCRETIONS					WHOLE SOIL WITHOUT LARGE CONCRE- TIONS
			12 mm.	6 mm.	4-5 mm.	2 mm.	<1 mm.	
C-4227	A_2	<i>inches</i> 2-6			17.0	24.8		
C-4228	A_2	6-10	8.4 9.4 9.2	13.3 10.9		25.4 17.8	15.2	4.3
C-4231	B	18-22				23.0	20.8	8.4

TABLE 6

Percentage of free iron oxide and total iron oxide in concretions from different horizons

LABORATORY NUMBER	HORIZON	DIAMETER OF CONCRETIONS	FREE Fe_2O_3	TOTAL Fe_2O_3
		<i>mm.</i>		
C-4227	A_2	4-5	16.8	17.0
C-4228	A_2	2	24.5	25.4
C-4231	B	2	20.0	23.0

mm.), however, is lower than that in the 2-mm. ones. The data, in general, are in agreement with those reported by Zakharov. They are, however, too meager for any definite conclusions regarding the iron content in concretions of similar size from different horizons. The percentage of Fe_2O_3 in the 2-mm. concretions from the A_2 and the B is about the same.

From the general appearance of the concretions, there is little doubt that the iron is present for the most part as the free oxide. For confirmation of this, the free iron oxide was determined on several samples of concretions by the sulfide method (13). The data, presented in table 6, show definitely that almost all the iron is present in the free oxide form.

Samples of the concretions treated with hydrogen peroxide effervesced vigorously, indicating the presence of considerable manganese dioxide. Also it was found that sulfur dioxide dissolved almost all the manganese from the concretions. This is added evidence that the manganese is present in the concretions as the dioxide or possibly as Mn_3O_4 .

GENERAL DISCUSSION

The data presented in tables 2, 3, 4, and 5 show an uneven distribution of iron and manganese throughout the horizons of the Dayton soil and a concentration of these elements in concretions. Such a concentration is particularly marked in the A_2 horizon, especially as regards manganese. The causes of this concentration and the formation of concretions are not yet known. It is almost certain that the cementation of soil particles in the form of concretions is produced by a segregation and precipitation, in certain spots, of iron and manganese oxides, but the causes of the mobilization and concentration of these oxides have not as yet been sufficiently explored. Tsukunaga (14) and Nikiforoff (5) have suggested the possibility of mobilization of iron and manganese by colonies of certain iron and manganese microorganisms.

Formation of iron-manganese concretions apparently is a typical feature of certain poorly drained soils which are subject to permanent or seasonal water-logging. Virtually all these soils are characterized by the strong development of glei. Much of the iron in these soils presumably is in the ferrous form, whereas the iron in the concretions is undoubtedly ferric. Local biochemical oxidation of ferrous and manganous compounds into the less soluble ferric and manganic compounds might be one of the causes of their fixation in the form of concretions.

Omeliansky (6) says:

There is a special group of bacteria, so called iron bacteria, which utilize comparatively large quantities of iron salts as a source of respiration. . . . Iron bacteria most frequently are present in bogs, ponds, and lakes, the water of which always contains ferrous iron, usually ferrous bicarbonate . . . in the poorly drained meadows this species might develop to such an extent that the water acquires a rusty color . . . Manganese, together with iron, is an important constituent in the life of the soil. Special microorganisms are responsible for the migration of this element in soil.

Waksman (16) points out that "various microorganisms are capable of extracting iron from solution and collecting it on their surface in the form of ferric hydroxide." He also refers to Beijerinck, who "described several fungi and bacteria which are capable of oxidizing manganese carbonates to oxides of manganese. Microorganisms also oxidize manganese salts of organic acid to manganese carbonates." Thiel (12), from an experimental study of the precipitation of manganese by microorganisms, concludes that "fungi that precipitate manganese from organic and inorganic salts of the metal are present in peat bogs, loamy and manganiferous soils and iron spring

slimes." He has found also that sulfate-reducing organisms grown under anaerobic conditions on solid culture media precipitate brown granules of manganese around the colonies and that various types of iron bacteria precipitate manganese as rapidly as iron.

It is possible that iron and manganese, after being oxidized and cemented into a solid grain, become unavailable for the microorganisms, and the developing colony, therefore, is forced to extract its supply of these elements from the surrounding media. It is also possible that a local precipitation of iron and manganese at the points of formation of the concretions disturbs the uniform concentration of these elements in the solution throughout the matrix, and the restoration of equilibrium results in movement of these compounds by diffusion toward the points at which the concentration has been reduced because of precipitation. In this way a concretion could grow until it was limited by the pore space or possibly a concentration of toxins terminated the development of the colony. If this is the case, then the concentrically layered or shelly structure of concretions reported by Tsukunaga and Winters can be explained by alternation, probably seasonal, of the periods of growth and of the dormant stages of the colonies.

It should be mentioned here that a large number of concretions from the Dayton soil were examined, but the concentric layers reported by other investigators were not very evident. Except for a thin outer coating, the concretions were either a uniform dark color or irregularly splotched with yellow and brown. An occasional concretion from the B horizon, however, did show a concentric layering, but this was not typical of the concretions in general.

W. O. Smith suggests¹ the possibility of segregation of iron and manganese from the solution, due to physical forces operating in the soil during its drying. According to his view (11), the large pores are freed of water first in the drying soil, and consequently the soil material that still remains saturated with water is broken into areas separated from each other by the drier soil with a more open pore space. As drying of the soil proceeds, the water front of each saturated area recedes toward the points inside this area with the smallest pore space. This can be accompanied by an increased concentration of the dissolved material, such as certain salts of iron and manganese. It might lead to an uneven distribution of these compounds throughout the soil and to an ultimate precipitation and oxidation of them in the form of concretions at the points of final desiccation of the soil. Added weight is given to Smith's suggestion by the fact that most concretions form in the A₂ horizon, which is waterlogged during the rainy seasons and dries during the dry summer periods.

Robinson (10) has found a high concentration of iron and manganese bicarbonates in the soil solutions of submerged soils. He attributes this to

¹ Private communication.

microbiological action on organic matter, which produces carbon dioxide along with other gases. In another paper (9) in discussing manganese dioxide in soils, he refers to several investigators who have found that manganese dioxide can absorb manganous salts from solution, changing them into the insoluble dioxide.

Taking the various suggestions and experimental data into account, the following hypothesis for the formation of concretions in Dayton soils is proposed: The soil solution under submerged conditions contains a high concentration of ferrous and manganous bicarbonates as a result of waterlogging and microbiological action on organic matter. As the soil dries, the iron and manganese is precipitated and oxidized to the oxides on the surface of the mineral grains at the points with the smallest pore space. The nuclei may be formed also through precipitation by microorganisms. Once the nuclei of ferric oxides and manganese oxides are formed, the concretions can grow from year to year by absorbing and oxidizing the ferrous and manganous salts at their surface.

Whatever the origin of the concretions, their formation indicates segregation of iron, manganese, and probably some other elements from the soil solutions. The composition of concretions in general may be unlike in different soils, but the general process of formation may be the same. This process can be regarded as a horizontal mobilization and segregation of certain compounds within the horizons. It can be contrasted with the process of vertical translocation from one horizon of the profile into another. The magnitude of segregation in the Dayton soil, especially that of manganese, indicates that in certain instances a horizontal mobilization may affect a large part of the mobile compounds in the soil. This process, therefore, should be given careful attention when the movement or translocation of soil components is considered.

In soils such as the Dayton or any other characterized by the presence of concretions of this type, the factors affecting horizontal mobilization apparently exert a greater influence than the factors of leaching, i.e., leaching or vertical translocation apparently is exceeded by the forces which tend to retain the mobile compounds within the horizons. If this is true, then eluviation and illuviation may not be the main processes leading to the development of the general profile.

In conclusion, it should be emphasized that if concretions greater than 2 mm. are removed from soils before analyses are made, the interpretations made from these analyses may be erroneous. The A₂ horizon of the Dayton soil, for example, contains 0.06 per cent of MnO after removal of concretions greater than 2 mm. The B horizon contains 0.15 per cent, which might indicate vertical translocation of the manganese. With all concretions present, however, the A₂ horizon contains 0.24 per cent MnO, and this gives an entirely different picture of the movement of the manganese.

SUMMARY

The distribution and composition of concretions in Dayton soils were investigated. These concretions range from about 0.05 to about 15 mm. in diameter and are present throughout the A and B horizons. The A₂ horizon contains the greatest number and the largest ones. In the profile examined in detail, over 11 per cent of the middle part of the A₂ horizon consists of concretions, most of which are greater than 2 mm. in diameter; the A₁ contains about 4 per cent, and the B about 3 per cent. Very few of the concretions in the B horizon are greater than 2 mm. in diameter. Firm concretions in the C horizon are rare.

The concretions contain a much higher percentage of iron and manganese, which are present as free oxides, than does the whole soil. The amount of Fe₂O₃ in the concretions ranges from about 8 to about 25 per cent, and MnO from less than 0.5 to almost 6 per cent. This is two to four times as much Fe₂O₃ and up to 75 times as much MnO as is contained in the soil.

The concentration of MnO is higher in the larger concretions than in the smaller ones and also higher in the lower horizons of the solum than in the upper.

The percentage of Fe₂O₃ in the smaller concretions is greater than that in the larger ones, except in the very small concretions (<1 mm.), which contain less iron than the slightly larger ones.

Certain biological, physical, and chemical factors which probably are involved in concretion formation are discussed.

The local segregation of MnO and Fe₂O₃ within each horizon is distinguished from the vertical migration from one horizon to another. The significance of this distinction relative to soil genesis is emphasized.

REFERENCES

- (1) GEMMERLING, V. V. 1922 Some data which characterized podzol soils. Russ. Pochvoved. No. 4-5. [Russian].
- (2) GLINKA, K. D. 1927. Pochvovedeni (Pedology). Moscow-Leningrad. [Russian].
- (3) JOFFE, J. S. 1936 Pedology. New Brunswick, N. J.
- (4) KOCHER, A. E., ET AL. 1917 Soil Survey of Yamhill County, Oregon. U. S. Department of Agriculture.
- (5) NIKIFOROFF, C. C. 1937 Soils of the phaneropodzolic group in western Oregon. *Soil Sci.* 44: 447-465.
- (6) OMELIANSKY, V. L. 1929 Soil Microbiology. Moscow-Leningrad. [Russian].
- (7) POPOV, T. T. 1914 Genesis and evolution of the aspen groves in Voronej province. *Trudy Dokuchaevskago Pochvennago Komitsta* 2: 1-172. St Petersburg. [Russian].
- (8) ROBERTS, R. C., ET AL. Soil survey of Puerto Rico. U. S. Department of Agriculture, (In press).
- (9) ROBINSON, W. O. 1929 Detection and significance of manganese dioxide in the soil. *Soil Sci.* 27: 335-350.
- (10) ROBINSON, W. O. 1930 Some chemical phases of submerged soil conditions. *Soil Sci.* 30: 197-217.
- (11) SMITH, W. O. 1936 Sorption in an ideal soil. *Soil Sci.* 41: 209-230.

- (12) THIEL, G. A. 1925 Manganese precipitated by microorganisms. *Econ. Geol.* 20: 301-310.
- (13) TRUOG, E., ET AL. 1936 Procedure for special type of mechanical and mineralogical soil analysis. *Proc. Soil Sci. Soc. Amer.* 1: 101-113.
- (14) TSUKUNAGA, K. 1932 Studies on the formation of iron concretions in Manchurian soils. *So. Manchurian Ry. Agr. Exp. Sta. Res. Bul.* 7:43-80.
- (15) TUMIN, G. M. 1912 Survey of the general character of soil morphology and its changes within the zones. *Zhur. Opytn. Agron.* 13: 321-353. [Russian].
- (16) WAKSMAN, S. A. 1932 Principles of Soil Microbiology, ed. 2. Williams and Wilkins, Baltimore.
- (17) WHEETING, L. C. 1936 Shot soils of western Washington. *Soil Sci.* 41: 35-45.
- (18) WINTERS, E. 1938 Ferromanganiferous concretions from some podzolic soils. *Soil Sci.* 46: 33-40.
- (19) ZAKHAROV, S. A. 1927 Kurs Pochvovedenia (A Course in Pedology). Moscow-Leningrad. [Russian].

DETERMINATION OF PORE-SIZE DISTRIBUTION IN SOILS¹

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It has been known for many years that the pore space of a soil directly controls the movement of air and of water in the soil, which in turn directly affects other soil properties and plant growth. The amount of percolation, and therefore of leaching and erosion, is related to the porosity of the soil, other factors being equal. No direct relationship has yet been found, however, between the total porosity and other soil properties or plant growth. In spite of this fact, the most common methods of measuring the pore space measure the total rather than the effective porosity.

Because of the relationship between the nature of the pores and the freedom of air and water movement in the soil, it would seem desirable to be able to measure the effective pore space as well as the total.

It has been the purpose of this investigation to develop a method by which the size distribution of the effective pores in the soil might be measured, and to study the relationship of this distribution to the rate of percolation of water through the soil. The method is a practical application of the theoretical discussion by Keen (15) of the relation of the capillary forces to the pore size.

Since this investigation was started (August 1937) some other papers (4, 8) which are closely related to the material presented here have come to our attention.

REVIEW OF LITERATURE

The most common method of determining the pore space of the soil is by calculation from specific gravity-volume weight determinations (18).

The method of determining the absolute specific gravity is unquestioned, but there is undoubtedly considerable error in the volume weight measurements. The most common methods of determining the volume weight, or apparent specific gravity, may be divided into two groups: those that measure disturbed soils, and those that measure soils in their natural state. The

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methods used in the first group obviously do not give a picture of the soils in their natural field condition. The methods in the second group are criticized because the pressure used to force the tube into the soil causes, especially in heavy soils, appreciable compaction of the soil ahead of the tube. This compaction is largely overcome by the use of cylinders of larger diameter (13).

In order to overcome the compaction of the soil which is encountered in the soil-tube method, the paraffin-immersion method was developed (23). Determinations at depths to 6 feet have been made by the use of an ordinary soil auger which removes a weighed amount of soil from a hole of measured volume. Various methods have been used in measuring the volume of the hole. Beckett (2) used a very viscous oil; Curry (7) used a screened, purified sand of known volume weight; Israelsen (13) inserted a very thin walled elastic-rubber tube into the auger hole and measured the amount of water required to fill the tube.

In an oven-dried soil the pore space will contain only air; under natural conditions, however, the pore space of the soil contains both air and water. The relative amounts of air and of water present will depend upon certain physical-chemical characteristics of the soil (1, 3, 10, 17, 19, 22). Chief among these are the texture, structure, and hygroscopicity. We know from general observation that a soil containing a large percentage of clay will tend to be only slightly permeable to air or water, whereas a sand will usually be open and well drained (10). Mathews (19) found that the permeability of the gumbo soil of the Belle Fourche irrigation project varied markedly with the initial water content and the structural condition of the soil. On the whole, it is safe to say that rapid percolation or good drainage goes parallel with coarse granular structure, although the dominant influence of this coarse structure can be entirely overcome by a very small amount of dispersed colloids, or other fine material, placed in certain positions (3).

The structure of the surface is not the only factor that influences the rate of percolation of water. In fine-grained soils which are underlain by a non-porous stratum, the surface may be sealed by rain water and air trapped in the profile. Continued percolation by capillarity then results in the development within the soil of an air pressure of considerable magnitude, and this pressure appreciably alters the rate of percolation. It follows that an impermeable lower horizon may be effective in retarding percolation before it has been reached by the percolating water (24). Lutz (17) observed that the physical nature of the B horizon, which is usually the limiting layer in permeability, determines largely the amount and rate of percolation.

Measurements of the rate of percolation of water through the soil profile, to be of any practical value, must be made on soils in their natural field condition. It has been found (24) that even field cores do not yield satisfactory results unless large numbers are studied under conditions that take into account the seasonal and other variations to which they are subject. Percola-

tion rates are of much importance because of the influence they have on the erosion of soil. Other things being equal, the soil having the higher rate of water penetration will have the lower amount of surface run-off, and consequently less erosion.

Because water movement must take place through the soil pores, the type, rate, and amount of movement will be related to the characteristics of the pores. According to the dominance of the moving force, the type of water movement may be divided into that which moves in the larger pores under the influence of gravity, and that which moves in the smaller pores under the influence of capillary forces. The rate of downward movement is determined by the size and characteristics of the pores. Slater and Byers (24) observed that the field passageways, such as root channels or structural cleavages, are more important than the character or volume of the pore space in determining the percolation rate.

In studying the relationship of the pore space to the rate of permeability, Dojarenko³ divided the pores into "capillary" and "noncapillary" pores, two terms suggested by Schumacher.³ The line of demarcation between the two sizes is, however, rather vague. The noncapillary pore space is defined as the sum of the volumes of the large pores which will not hold water by capillary forces but which will permit the percolation of water; and the capillary pore space, as the sum of the volumes of the small pores that hold water by capillary forces. The sum of these two is obviously the total pore space. The capillary pore space is determined by measuring the amount of water that a soil sample will absorb when in contact with a saturated filter paper or other such material. Dojarenko assumed that, for optimum plant growth, 50-55 per cent of the total pore space should be noncapillary under the conditions prevalent in the moist regions of Russia; whereas Kwasnikow³ calculated that it should be between 35-40 per cent for the black-earth soils. Krause (16) has summarized the condition by saying that "By exceeding a certain minimum noncapillary pore volume the biological activity of the soil expires from a lack of air and by exceeding a maximum it dies from a lack of water."

The capillary pores do not have a significant influence on the rate of infiltration, although there is some water movement through them. They are of extreme hydrological importance, however, in that they are responsible for the water retained in the soil after the excess mobile water has drained away.

Green and Ampt (10) and others have found that with certain limitations, both the Poiseuille and the Meyer-Poiseuille formulas for capillary flow of liquids through capillary tubes can be applied to soils. Their calculations were made, however, on soils saturated with water, a condition which is found only when dealing with the downward flow of water after a heavy rainfall or

³ Cited by Krause (16).

following irrigation. Normally, air occupies the noncapillary pores, water the capillary.

In order to eliminate some of the many variable and complex factors influencing the movement of water in a soil, an "ideal soil" has been used for study (15). "Glistening Dew," a trade product consisting of minute spheres of considerable uniformity, has been found to be as satisfactory as anything available. As it is impossible to obtain perfect arrangement in any random packing, it may be assumed that the effective pore space, in a sufficiently large cross-section, is essentially continuous and of practically uniform dimensions.

When a system of uniform spherical particles is placed in contact with a free water surface, the water spreads over the surface of the lower particles, and wherever two particles come into contact, a ring of water which takes the shape of a wedge is formed. The surface tension of the water tends to reduce the free surface to a minimum by drawing in more water from the adjacent particles. As more water is attracted to the wedge, the rings around the point of contact so enlarge that they join to form a triangular pore which will tend to be reduced in diameter until the pore is entirely filled, provided its dimensions are not too great. The net result of this process, which takes place at numerous points of contact throughout the mass, is to draw water from adjacent regions, and, by successive displacement, eventually from the ground water itself. Equilibrium is established when the pressure head is sufficient to balance the soil moisture tension. This combination of the simple capillary-tube hypothesis and the one originated by Briggs (5) is reported by Keen (14) as depicting the mechanism of water ascent in the ideal soil. The whole process is based on the fact that liquid tends to reduce its free surface energy and, consequently, its exposed surface, to a minimum.

In a soil which contains particles of varying size, there will be a corresponding variation in the effective dimensions of the pore spaces. With this in mind, it is evident that a soil column can be regarded as a collection of capillary tubes distributed over a certain range of effective diameter. Thus, if the texture of the soil is not too coarse, one can expect to find in successive planes above the ground water level, (a) complete saturation, (b) complete saturation of smaller pores and incomplete saturation of the larger ones, and finally, (c) a region of incomplete and decreasing saturation in the smaller pores. This would result in a decrease in the moisture content with height above the water level, a condition which has been reported several times (9, 11, 12, 14, 22).

The same fundamental forces are effective in an actual soil that are active in an ideal soil. Briggs (5) pointed out many years ago that the underlying physical factors concerned in the capillary movement of moisture are the surface tension and the coefficient of viscosity of the liquid, together with the geometrical configuration of a rather complex three-phase soil mass.

From the foregoing discussion, it appears that permeability would be af-

fectured by the fluid used and also by the extent to which the capillaries are modified by adsorption of the fluid. Green and Ampt (10) presented data which show that modification does occur probably in two ways: (a) by the moisture in the soil restricting the area of the capillaries through which the air is passing, and (b) by the swelling of the humus, clay, or other colloidal material in the soil when wetted. It is evident that any increase in the volume of the soil mass due to swelling will result in a decrease in the air capacity of the soil. As the swelling increases, and as the films of moisture over the particles become thicker and thicker, they are held less and less tightly. If a film is subjected to an increase in pressure, it will tend to lose water to that portion of the soil where a lower pressure is exerted by surface forces. If there were no loss by evaporation from either section of soil, the forces acting on the two would soon come to equilibrium and no more water movement would take place. It is not necessary for two portions of a soil to contain the same amount of water to be in equilibrium; for example, a sand will lose water to a clay soil which has much more moisture as expressed on a percent-by-weight basis. In studying the tensions developed by these forces in soils, Richards (20) has used the term "capillary potential." Capillary potential differences have the same relation to the flow of moisture in the soil as voltage differences have to the flow of electricity in wires, or as pressure differences have to the flow of water in pipes.

The method to be described for determining pore size is based on the capillary forces in the soil.

PROCEDURE

In his theoretical discussion, Keen (15) suggested the use of a Büchner funnel connected to a burette-manometer to measure the pore size of a soil. By adjusting the height of water in the burette in small steps, a given series of suction values, or pressure deficiencies, could be produced. By noting the changes of volume of water in the burette, it was possible to follow changes in moisture content with changes in suction. This setup may be satisfactory for very low tensions; but it was found that, at higher tensions, the larger pores allowed air to pass through the soil mass, and the tension could not be maintained. To prevent this free movement of air through the larger pores, the soil was placed on a porous plate. This plate was sufficiently porous to permit water to move through it freely, but the pores were sufficiently small to prevent the water from being sucked out of them until a relatively high tension had been reached. In principle, the apparatus as finally used bears a close resemblance to that described by Richards (20).

It was found advisable to use a suction pump to obtain the tensions in preference to differential leveling of water columns, because of the height to which the levels would have to be raised. This change necessitated the introduction of a control valve by which the tensions could be maintained at any desired level. Here again a modification of an apparatus suggested by Rich-

ards (21) was used. A section of a Pasteur-Chamberland filter (*A*, fig. 1) was inserted in the mantle of a Berkfeld filter (*B*) and connected through the ends by means of glass tubing and rubber stoppers. The tube from one end was connected to a closed-tube manometer (*C*), the other, to the burette (*D*), which in turn was connected into the base (*E*) of the apparatus. A tube from the glass mantle was connected to a water suction pump (*F*), which was controlled automatically by means of a solenoid valve (*G*). Because it was necessary to change the amount of mercury in the closed-tube manometer for different pressures, it was found desirable to read the pressures on another manometer (*H*).

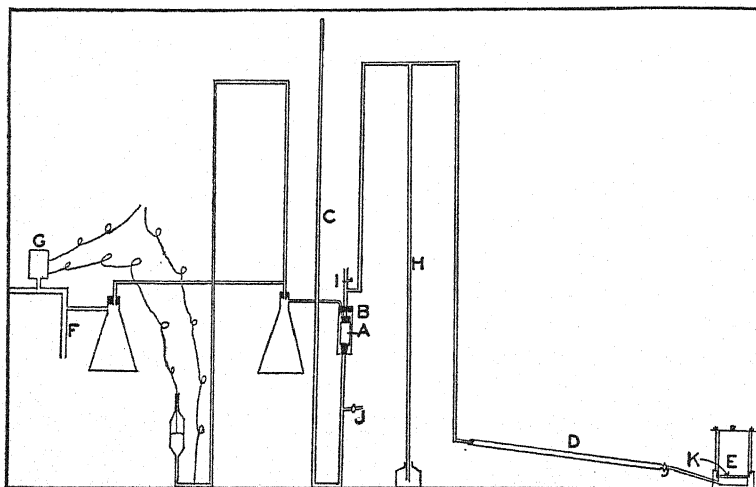


FIG. 1. DIAGRAM OF THE APPARATUS USED FOR DETERMINING PORE-SIZE DISTRIBUTION

For low tensions, mercury was added through the T-tube at the top of the automatic valve (*I*); and for higher tensions, it was removed through a stopcock (*J*). When air was removed from the glass mantle, a pressure gradient was built up between the pressure in the system and that in the mantle. This pressure gradient tended to be reduced by the movement of air from the rest of the system through the porous cylinder into the mantle. As the air was removed from the system, the mercury rose into the porous cylinder until it was entirely filled. At this point, as no more air could escape through the porous cylinder, the system remained at the desired tension. If air leaked into the system, the mercury fell in the clay cylinder, and the above process was repeated. This system was found to be very satisfactory, as it was possible to limit the variation from the desired tension to 1 or 2 mm. of mercury.

The burette was placed in the near-horizontal position in order to eliminate the necessity of correcting the tension for every increment of change in the amount of water in the burette.

A continuous water connection was made from the water in the burette to

the water in the soil through the base (*E*) of the apparatus and the porous plate (*K*).

Samples were collected in their natural field condition by using a soil-sampling tube similar to the one described by Coile (6). It was found that the most satisfactory results were obtained if the tube was forced into the soil horizontally by placing an automobile jack against the opposite side of a hole of the depth of the desired sample. As the samples were collected, the cylinders containing them were placed in quart ice-cream containers, taken to the laboratory, and weighed.

At the start of a determination the cylinder and sample were reweighed and clamped in the base of the apparatus, and the sample was saturated from the bottom. In order to facilitate the saturation, it was found advantageous to apply a tension of about 6 inches of mercury to the top of the sample; in the sand samples a lower tension was more desirable. When the sample was completely covered with water, the suction was released, the sample was allowed to drain until just saturated, and the amount required for saturation was noted. The meniscus in the burette was then set at the 100-cc. mark, and the regulator was set for a very low tension. As soon as the soil had come to equilibrium with this tension, as evidenced by a constant burette reading, the amount of water removed was recorded and the tension increased. This process was repeated in steps of increasing tensions until air began to be sucked through the soil into the system. The sample was then removed from the apparatus and weighed. The amount of water remaining in the soil was determined by drying at 105° C. and reweighing. The plate in the apparatus was allowed to saturate again, and the total amount of water removed from the soil was determined by the final burette reading.

The volume of the cylinder not occupied by the soil was determined by pouring a weighed amount of mercury on the top of the sample and then measuring the amount of water required to fill the cylinder. Subtracting this from the volume of the cylinder gave the volume of the sample. It was found that, in compact samples, it was possible to pour the mercury on top of the soil; but with the more open samples, as the mercury would enter the larger pores or cracks, it was necessary to use a very thin sheet of rubber to cover the soil surface.

In order to eliminate the complicating factors introduced by the colloidal material in actual soils, pure sand was separated into the various-sized groupings for testing the apparatus, the theory, and making the initial determinations.

RESULTS

The first determinations were made on sand separates. Each separate was placed in the sampling tube of the apparatus, saturated from the bottom, and a determination made according to the procedure outlined for soils. The results obtained are shown in figure 2. The coarse sand was found to be too coarse to give satisfactory results.

In all cases, the amount of water required to saturate completely the sample from the oven-dry condition was taken as the total pore space of the sample. This method gave results which checked to within 0.6 per cent of the results given by the specific gravity-volume weight method. The volume weight was determined after the porosity determinations had been made as outlined.

All calculations in this work are based on the capillary-rise formula, $h = \frac{2s}{r p g}$, which becomes $d = \frac{3}{h}$, where d is the diameter of the pore in millimeters and h is the amount of tension expressed in centimeters of water with which soil water is in equilibrium. By the use of this formula, it is possible

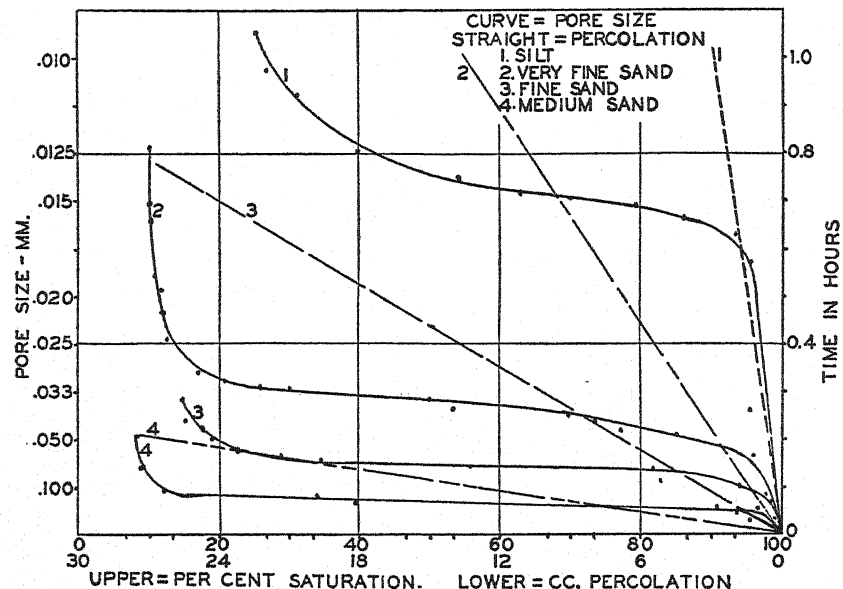


FIG. 2. SOIL MOISTURE TENSION AND PERCOLATION CURVES FOR THE SEVERAL SOIL SEPARATES

to calculate the diameter of the pores emptied by any given tension. Measuring the volume of water removed from the soil by any given tension gives the volume of pores of the size indicated by that tension. The calculations are facilitated by plotting the tension as a function of the percentage saturation with water. The volume of pores between two sizes can be calculated from the volume of soil core and the degree of saturation at the two tensions. By this procedure, it is possible to determine the size distribution of the pores which are effective in allowing percolation.

The striking result to be noticed about these sand curves is that the largest percentage of the water was removed from any one sample over a very small range of tension. This fact indicates great uniformity in the diameters of

the pores in these materials. The slope of the curve increases slightly as the fineness of the material increases, indicating a greater range of pore diameters as the material becomes finer. The individual pores, however, become smaller as the material becomes finer. The actual distribution is shown in figure 3. By making a permeability determination on each of these materials, a close connection was found between the sizes of the pores and the permeability. The medium sand allowed water to percolate through it at a rate equivalent to 175 inches per hour; the fine sand, 53.1 inches per hour; the very fine sand,

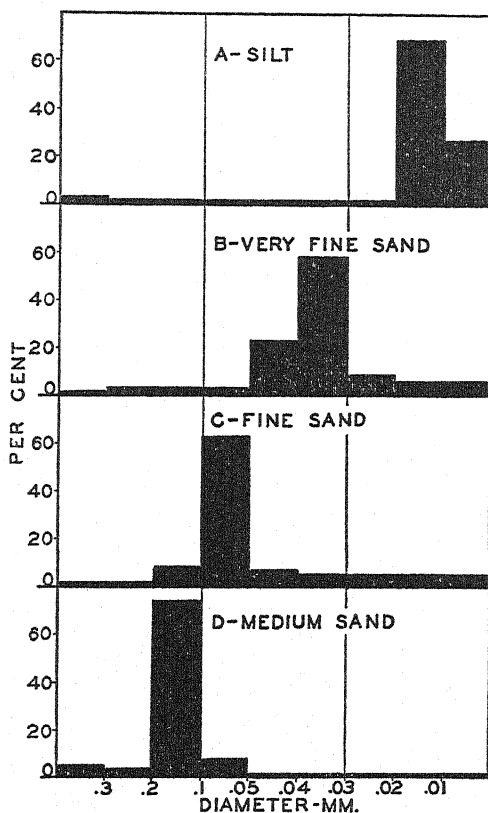


FIG. 3. PORE-SIZE DISTRIBUTION IN THE SEVERAL SOIL SEPARATES

13.4 inches per hour; and the silt only 2.95 inches per hour. This relationship is shown in figure 2.

In order to show the differences between soil types, determinations were made on a Cecil clay subsoil obtained near Raleigh, North Carolina, and on an Iredell clay subsoil from a near-by area. The data for these two samples are presented in figure 4.

It will be seen from these curves that the Iredell has a very small amount of pore space large enough to be drained by the tensions capable of being

developed by the apparatus. On the other hand, 50 per cent of the pore space of the Cecil was shown to be larger than 0.021 mm. in diameter. This difference between the two soils is apparent in the characteristics of the soils under natural conditions. The Iredell is recognized as a very impermeable soil. Laboratory determinations on the permeability of the Iredell subsoil showed that no water percolated through the sample under a head of half an inch of water; it was found that a tension of 6 inches of mercury was necessary to draw water through the sample in any appreciable amounts; and even then, the movement was very slow. Similar determinations made on the Cecil showed that it permits 1.86 inches of water to percolate per hour under a

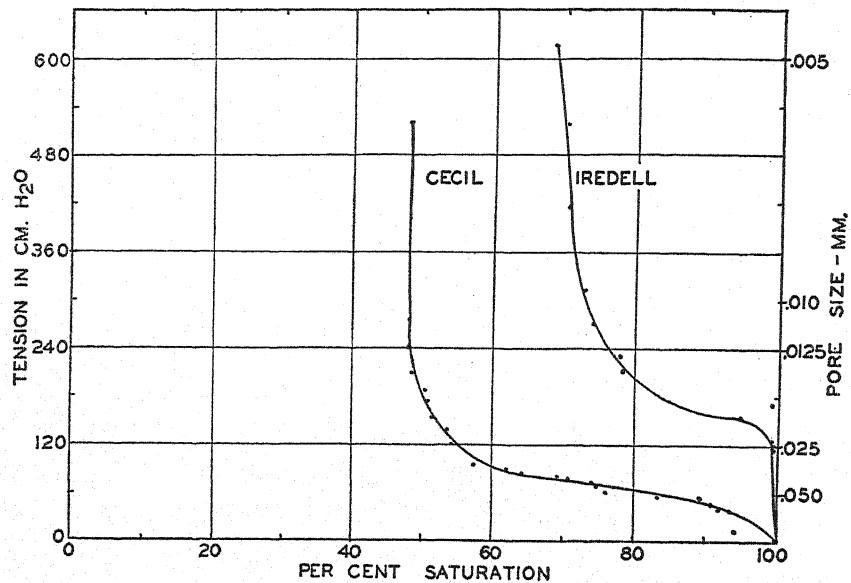


FIG. 4. SOIL MOISTURE TENSION CURVES OF CECIL AND IREDELL SUBSOILS

head of half an inch of water. This is in agreement with field observations, for the Cecil is fairly well drained and well aerated.

DISCUSSION

Other than external forces, which are considered constant in this discussion, the forces affecting water in the soil may be grouped into two classes: (a) those exerted by the soil particles on the water, and (b) those within the water itself.

The first group includes those forces which attract the water to the soil particles and tend to prevent its free movement from one place in the soil to another. Because the measurements dealt with in this paper, however, are of effective rather than total porosity, these forces, and the water held by them, are concerned only indirectly.

The important forces in the second group are surface tension and viscosity. Under isothermal conditions, these forces will not change in the soil unless some external factor is brought to bear upon the water. If, therefore, external factors can be controlled, it is possible to apply the capillary-rise formula to the water in the soil, since the same forces are active in the soil that are active in capillary tubes. Richards (20) pointed out that if capillary tubes of various diameters were sealed in the same system as a soil prism, when equilibrium existed the water in the soil must have the same surface curvature as it would have in a capillary tube at the same height and at the same diameter. Otherwise, there would be a difference in the vapor pressure at the two points, and the consequent distillation of the water would result in a cyclic motion of water, a condition which is impossible according to the second law of thermodynamics.

Water exists in soil as thin films spread over the surface of the soil particles and in rings at the points of contact between two or more particles. These rings are exposed to the soil atmosphere in much the same manner as the surface of a liquid is exposed in a capillary tube. The surface tension of the liquid tends to reduce the free energy, and hence the surface exposed, to a minimum; consequently, if there is a supply of water in contact with an unsaturated soil there will be a pressure gradient between the surface of the water and the surface of the rings around the points of contact in the soil. This pressure gradient will tend to draw water from the free water surface into the soil. This attractive force is commonly spoken of as capillary pull.

This capillary pull may be counteracted by reducing the pressure on the free water surface. When this pressure is so reduced that the tension on the free water surface equals that of the surface tension of the water in the soil, there will be no movement of water. If the tension on the free water surface is greater than that exerted by the water in the soil, there will be a movement of water from the soil to the free water.

In this procedure, the pressure deficit, or tension, applied to the surface of the free water was regulated and the amount of water withdrawn from the soil at each tension was measured. This gives the amount of pore space in the soil that has the power to hold water with a given force. The power to attract water, as has just been pointed out, depends upon the curvature of the surface of the water in the soil, which is in turn dependent upon the size of the pores in the soil.

It is evident from this that the amount of pore space reported with each reading is the amount emptied at each tension; consequently, the volume of the pores reported is the effective volume. It is believed that water held by the soil particles by forces other than surface tension is not free to move, and consequently has the effect of increasing the size of the soil particles, and hence reducing the effective pore space.

It is also evident that the height of tension applied approximates the height to which water will rise in the soil by true capillarity. The observed heights

of capillary rise do not agree with this in all cases, because the observed rise is not due entirely to capillary forces; the attractive forces of the soil play a part as important as, or more important than, the true capillary forces.

Soils with a wide range of physical properties were chosen for these investigations. They varied from a group of sand separates to the plastic, sticky subsoil of the Iredell. A silt separate and Cecil subsoil and topsoils were used to represent the intermediate group. Because of the absence of many complicating factors encountered in soils, the sands were chosen as a convenient material with which to calibrate the apparatus and to determine the accuracy of the method. Furthermore, they are representative of large areas of soils.

The Iredell and the Cecil were chosen as representatives of soils with heavy clay subsoils which predominate throughout the Piedmont Plateau of North Carolina. The subsoils of both have a high clay content, the Cecil probably having slightly more than the Iredell. The total porosity is approximately the same in each. Because of these similarities, it would be expected that both soils would have similar internal drainage properties. This, however, is contrary to field observations. The Iredell has a wet, plastic, sticky subsoil which is very impermeable to water and air. The Cecil, on the other hand, has a compact, brittle subsoil which is permeable to water, but much less so than the sand or silt separates, even though the latter contain a lower total porosity.

These observations lead to the obvious conclusion that percolation and aeration in soils are dependent upon the size rather than the amount of pore space, and that not all soils, even those of the same mechanical composition, apparently have the same sized pores.

The data presented showed this to be true. The sands had large pores and, therefore, a low capacity to hold water. Such soils have excessive internal drainage, as evidenced by the percolation data and by field observations. This is especially true if the sandy layer is deep as in the sandhill area of North Carolina. In such soils, moisture conservation becomes a major problem.

Contrasted with the sands is the Iredell soil of the Piedmont. Field observations show that this soil is very impermeable to water; therefore, it has a high run-off loss and, consequently, is seriously eroded. It was found that a tension of 6 inches of mercury was necessary to pull water through a column about 3 inches long. This is equivalent to a head of 81 inches of water. Such a resistance to percolation indicates that the pores are very small. Measurements showed that 75.5 per cent were less than 0.012 mm. in diameter. Lutz (17) has shown that Iredell clay adsorbs fairly large films of water, which undoubtedly reduce the effective size of the pores to such an extent that an 81-inch head of water is necessary to overcome the forces holding these films.

Between the two extreme conditions, as represented by the sands and the Iredell, are many soils, of which the Cecil is one. The Cecil subsoil has a high clay content, but, unlike that of the Iredell, it is not hydrated to any appreciable extent. The pores, therefore, are not clogged with film water.

They are, however, rather small, and the water movement is slow. Granulation of the subsoil into aggregates similar to those found in the Davidson would be more desirable; the soil would then have enough large pores between the aggregates for aeration, and enough small ones within them to hold a sufficient supply of moisture.

The optimum size distribution of pores is not known, but obviously it must be somewhere between that of the sands and the Iredell. Russian workers (16) have reported that 35 to 50 per cent of the pore space should be of non-capillary size; however, they have not clearly defined the limits of the non-capillary pores.

It is very evident that more work must be done before any definite conclusions can be drawn concerning the optimum size distribution of soil pores. It is believed, however, that this method will throw much light on the problem of soil permeability. When used together with percolation determinations, it should lead to a much better understanding of soil structure than has previously been possible to attain.

SUMMARY AND CONCLUSIONS

A method has been developed whereby the size distribution of the pores in the soil may be measured by applying tensions equal to the capillary tensional forces developed in the soil. The tensions were automatically controlled at any given value, and the amount of water removed from the soil was measured in a burette.

The capillary pull may be counteracted by reducing the pressure on the free water surface. When this pressure is so reduced that the tension on the free water surface equals that of the capillary tension of the water in the soil, there will be no movement of water. If the tension on the free water surface is greater than that exerted by the water in the soil, there will be a movement of water from the soil to the free water.

All calculations are based on the capillary-rise formula. This is possible because the water in the soil is subject to the same surface tensional forces which are active in capillary tubes. According to the second law of thermodynamics, when equilibrium exists in the soil, the water in the pores must have the same curvature as it would have in a capillary tube of the same diameter.

Permeability studies show that percolation and aeration in soils are dependent upon the size rather than the amount of pore space, and that not all soils, even those of the same mechanical composition, apparently have the same sized pores.

The optimum size distribution of pores is not known, but it is believed that this method will throw much light on the problem of soil permeability and structure.

The following conclusions may be drawn from the results:

The method for determining the pore-size distribution is believed to be as good as any yet developed for studying soil porosity.

There is no relationship between the total porosity and the effective pore space.

There is a direct relationship between effective pore space and permeability.

Further studies should make it possible to determine whether certain crops such as tobacco are adapted to specific soils, and, if drainage is necessary, what type and what spacing are required.

REFERENCES

- (1) BAVER, L. D. 1933 Soil porosity as an index of structure. *Amer. Soil Survey Assoc. Bul.* 14: 83-87.
- (2) BECKETT, S. H. 1928 The use of highly viscous fluids in the determination of the volume weight of soils. *Soil Sci.* 25: 481-483.
- (3) BOUYOUCOS, G. J. 1930 A new method of measuring the comparative rate of percolation of water in different soils. *Jour. Amer. Soc. Agron.* 22: 438-445.
- (4) BRADFIELD, R., AND JAMISON, V. C. 1938 Soil structure—attempts at its quantitative characterization. *Proc. Soil Sci. Soc. Amer.* 3: 70-76.
- (5) BRIGGS, L. J. 1898 The mechanics of soil moisture. U. S. Dept. Agr. Bur. Soils Bul. 10.
- (6) COILE, T. S. 1936 Soil samplers. *Soil Sci.* 42: 139-142.
- (7) CURRY, A. S. 1931 A comparison of methods for determining the volume weight of soils. *Jour. Agr. Res.* 42: 765-772.
- (8) DONAT, J. 1937 Das Gefüge des Bodens und dessen Kennzeichnung. *Trans. Sixth Comm. Internat. Soc. Soil Sci.* B: 423-439.
- (9) GARDNER, W. 1919 The movement of moisture in soil by capillarity. *Soil Sci.* 7: 313-317.
- (10) GREEN, H., AND AMPT, G. W. 1911 The flow of air and water through soils. *Jour. Agr. Sci.* 4: 1-24.
- (11) HARDY, F. 1927 The measurement of "suction forces" in colloidal soils. *Soil Sci.* 24: 71-75.
- (12) HARRIS, F. S., AND TURPIN, H. W. 1917 Movement and distribution of moisture in the soil. *Jour. Agr. Res.* 10: 113-155.
- (13) ISRAELSEN, O. W. 1918 Studies on capacities of soil for irrigation water and on a new method of determining volume weight. *Jour. Agr. Res.* 13: 1-35.
- (14) KEEN, B. A. 1912 On the moisture relationships in an ideal soil. *Jour. Agr. Sci.* 14: 170-177.
- (15) KEEN, B. A. 1931 The Physical Properties of the Soil. Longmans, Green and Co., New York.
- (16) KRAUSE, M. 1931 Russian investigations in the field of soil structure. *Landw. Jahrb.* 73: 603-690.
- (17) LUTZ, J. F. 1934 The physico-chemical properties of soils affecting soil erosion. Missouri Agr. Exp. Sta. Res. Bul. 212.
- (18) LYON, L. T., AND BUCKMAN, H. O. 1935 The Nature and Properties of Soils. The MacMillan Co., New York.
- (19) MATHEWS, O. R. 1916 Water penetration in gumbo soils of Belle Fourche reclamation project. U. S. Dept. Agr. Bul. 447.
- (20) RICHARDS, L. A. 1928 The usefulness of capillary potential to soil moisture and plant investigators. *Jour. Agr. Res.* 37: 719-742.
- (21) RICHARDS, L. A. 1931 Low vacuum pressure control apparatus. *Rev. Sci. Instruments* 2: 49-52.
- (22) SCHOFIELD, C. S. 1924 The movement of water in irrigated soils. *Jour. Agr. Res.* 27: 617-693.
- (23) SHAW, C. F. 1917 A method for determining the volume weight of soils in field condition. *Jour. Amer. Soc. Agron.* 9: 38-42.
- (24) SLATER, C. S., AND BYERS, H. G. 1931 A laboratory study of the field percolation rates of soils. U. S. Dept. Agr. Tech. Bul. 232.

IODINE CONTENT OF SOME TEXAS SOILS

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Correlated field and chemical studies of the relation of fertilizers, tillage, and crop residues to the incidence of cotton root rot by the Division of Soil Fertility Investigations, Bureau of Plant Industry, have been in progress in Texas during a period of years. These have been confined largely to the Blackland prairie section where the root-rot disease is widespread and has become a limiting factor in the economical production of cotton and other susceptible crops. The disease is caused by a soil-inhabiting fungus *Phy-matotrichum omnivorum* (Shear) Duggar (9).

The early laboratory program² dealt with the composition of the soils of the section as a possible factor influencing the incidence of the disease and with the relation of fertility to the problem in general. Special attention was given to some of the less abundant elements, particularly iodine. Less information is available on the iodine content of soils than on that of plant and animal tissues. The relation of iodine to human and animal nutrition makes these data of interest.

SOILS STUDIED

Figure 1 shows the location of the Blackland prairie section within the state and the locations of the areas from which samples were collected. The most prevalent soils are those of the Houston series, chiefly black clay and clay types, which, according to Carter (3), cover approximately 80 per cent of the total upland area of the section. The soils of the Wilson series, including the clay and clay loam types, are second in extent. The terrace correlative for the Houston is the Bell, and for the Wilson the Irving series. The Houston soils are known for their productivity, which has continued at a relatively high level despite widespread continuous cropping to cotton.

In addition to the soils of the Blackland prairie, data are reported for a sample of Denton clay, an important soil of the Grand prairie section that

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² The early studies were developed under the direction of J. J. Skinner, in charge of cotton soil and fertilizer investigations, with P. R. Dawson, then in charge of the soil fertility cotton root-rot investigations at Austin, Texas. Acknowledgment is made to J. E. Adams, now in charge of the Austin station, for aid in the preparation of the manuscript.

merges with the Blackland prairie section at points on its western margin; two samples of Yahola silty clay loam, an alluvial soil of the Brazos River Valley, which transects the major Blackland prairie section in McLennan and Falls Counties; a sample of Victoria clay, a soil of the Rio Grande plain; and two samples of Harlingen clay, a heavy alluvial soil of the Lower Rio Grande Valley. These soils are described in detail by Carter (3).

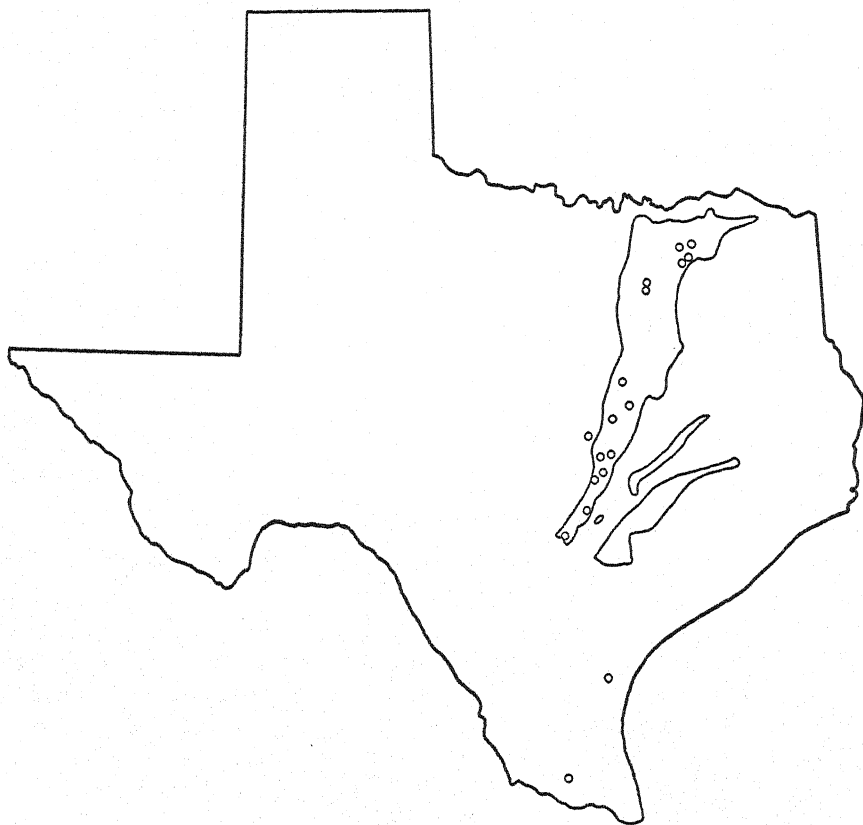


FIG. 1. OUTLINE MAP OF TEXAS SHOWING THE BLACKLAND PRAIRIE SECTION (CIRCUMSCRIBED AREAS) AND LOCATIONS SAMPLED (CIRCLES)

In most cases the areas sampled were those employed for field experiments, on which observations and records of crop response and root-rot distribution extending over several years are available. In other cases the fields were under frequent observation. In practically all instances, each sample comprised four subsamples from nonfertilized areas taken at arbitrary levels of 0-6, 6-12, 12-24, and 24-36 inches. Pertinent descriptive data relating to each sample are summarized in table 1 with the analytical data.

TABLE 1
Iodine content of some Texas soils
 In p.p.m. air-dry soil

SAMPLE NUM- BER*	SOIL TYPE	LOCATION	SOIL CHARACTERISTICS	DEPTH	IODINE CONTENT	
					Nonin- fested to slightly infested root-rot soils	Heavily infested root-rot soils
				<i>inches</i>		
17, 16	Houston black clay, flat phase	Dallas County; experi- ment field on A. J. King farm	Heavy black calcareous clay of level topography; irregularly infested with cotton root rot	0-6	3.226	4.396
				6-12	3.520	4.989
				12-24	4.693	3.226
				24-36	2.346	5.866
73	Houston black clay	Dallas County; experi- ment field on W. E. Jones farm	Heavy black calcareous clay of slightly rolling topography; extensive area entirely free of root rot	0-6	3.226
				6-12	2.347
				12-24	2.640
				24-36	5.280
88	Houston black clay	Bell County; near Temple	Heavy black calcareous clay of rolling topography; virgin soil in grass	0-6	4.106
				6-12	10.550
				12-24	7.333
				24-36	7.626
39	Houston black clay, flat phase	Travis County; experi- ment field on W. F. Voelker farm	Heavy black calcareous clay of level topography; predomi- nantly infested with root rot	0-6	6.057
				6-12	4.956
				12-24	2.753
				24-36	1.101
110, 105	Houston black clay, flat phase	Caldwell County; ex- periment field on Blanks Plantation	Heavy black calcareous clay; level topography, negligible erosion, highly productive	0-6	16.520	15.890
				6-12	18.720	12.910
				12-24	18.150	7.626
				24-36	22.030	9.679
327, 328	Houston clay loam	Bexar County; plats of U. S. San Antonio Field Station	Black clay loam of level topog- raphy; predominantly heavily infested with root rot	0-6	7.434	6.608
				6-12	8.921	7.599
				12-24	7.764	4.224
				24-36	6.278	10.070
325, 326	Houston black clay	Hunt County; plats of U. S. Cotton Breed- ing Field Station	Very heavy and tight black clay, noncalcareous at surface ex- cept for small local spots	0-6	6.057	6.057
				6-12	4.956	6.057
				12-24	4.405	5.507
				24-36	7.709	4.405
167	Wilson clay	Hunt County; experi- ment field on A. K. Foster farm	Brownish gray clay loam; cal- careous, shallow phase soil; heavily infested with root rot	0-6	4.396
				6-12	3.226
				12-24	4.693
				24-36	4.693
168	Wilson loam	Hunt County; experi- ment field on A. K. Foster farm	Dark gray clay loam; noncalcar- eous and slightly acid deep- phase soil from same field as No. 167. Less severely infested	0-6	0.587
				6-12	1.174
				12-24	0.880
				24-36	2.347
45, 46	Wilson clay loam	Hunt County; experi- ment field on R. W. Craig farm	Brownish gray clay loam (No. 46) deep-phase, slightly acid at surface; infested (No. 45) shallow phase calcareous soil, severely infested	0-6	2.029†	2.817
				6-12	6.325†	2.930
				12-24	5.866	4.989
				24-36	2.933	5.280

TABLE 1—*Concluded*

SAMPLE NUM- BER*	SOIL TYPE	LOCATION	SOIL CHARACTERISTICS	DEPTH	IODINE CONTENT	
					Nonin- fested to slightly infested root-rot soils	Heavily infested root-rot soils
				<i>inches</i>		
230, 242	Irving clay	Williamson County; O. Nelson farm	Heavy gray-black clay, a terrace correlative of Wilson clay. Root rot associated with cal- careous spots	0-6	10.270	4.989
				6-12	4.106	7.626
				12-24	5.280	4.693
				24-36	11.150	5.280
234				0-6	4.693
				6-12	8.799
				12-24	8.799
				24-36	8.213
329	Denton clay, light colored phase	Williamson County; experiment field on A. Peterson farm	Brown friable calcareous clay, slightly rolling topography. Parent material at depth of 20-30 inches	0-6	22.030
				6-12	20.930
				12-24	23.130
				24-36	7.710
25, 24	Yahola silty clay loam	McLennan County; experiment field (1929) on Earl Farm, Brazos River Bottom	Brownish red, calcareous, silty clay loam, alluvial. Only lo- calized areas infested with root rot	0-6	0.125	0.253
				6-12	0.587	0.587
				12-24	1.174	1.174
				24-36	2.640	1.174
76	Victoria clay	Nueces County; near Corpus Christi	Heavy black calcareous clay; level topography. From ex- tensive area free of root rot
				6-12	6.453
				12-24	12.610
				24-36	11.730
59, 58	Harlingen clay	Hidalgo County; ex- periment field on Freeman farm	Heavy, brownish gray, calcare- ous, alluvial clay	0-6	2.347	0.880
				6-12	2.053	3.520
				12-24	3.226	4.693
				24-36	2.053	3.226
174	Wilson clay	Travis County; Kruger farm	Brownish gray clay loam, slightly acid in surface 6 in- ches; subsoil becomes increas- ingly calcareous with depth	0-6	2.913
				6-12	3.045
				12-24	4.236
				24-36	4.243
175	Wilson clay	Falls County; Davis farm	Brownish gray clay loam with noncalcareous surface to 6- inch depth. Calcareous sub- soil	0-6	4.243
				6-12	6.994
				12-24	2.973
				24-36	8.213

* Samples taken and analyzed from 1932 to 1933.

† 0-2 inches.

‡ 2-12 inches.

EXPERIMENTAL PROCEDURE

The method used for the determination of iodine embodies the essential features of the official method of the Association of Official Agricultural Chemists (2, p. 8-9) and that of Norris and Rao (6).

The procedure is as follows: 15-50 gm. of 100-mesh air-dry soil is heated for 2 hours at 1000-1100°C. in a silica tube in a stream of oxygen passed through 5 per cent KOH; the evolved gases are absorbed in a series of three bottles

containing a saturated solution of $\text{Ca}(\text{OH})_2$ with a slight excess in suspension. The contents of the absorption train are transferred to a beaker and heated on a steam bath for 30 minutes, filtered, and washed with hot water. The filtrate and washings are evaporated to dryness in a 30-cc. casserole, 2 cc. of $\text{Ca}(\text{OH})_2$ added and again brought to dryness, and the temperature increased to char any organic matter present. The residue is extracted with small portions of hot water (total volume used about 20 cc.) and filtered into a 50-cc. glass-stoppered bottle, acidified with 1:9 H_2SO_4 , and a small amount of NaNO_2 added. After vigorous shaking, the liberated iodine is extracted with 1-cc. portions of colorless CS_2 ; two extractions usually being adequate. The CS_2 extract is collected on a hard filter paper, washed twice with a small amount of water, and transferred to a 30-cc. glass-stoppered bottle. After addition of 2 cc. of saturated sodium acetate solution, titration is with 0.001 N $\text{Na}_2\text{S}_2\text{O}_3$ by drop-wise additions until the pink color is discharged. Vigorous shaking, after each addition of $\text{Na}_2\text{S}_2\text{O}_3$, is necessary for a rapid reaction. Calculation is made to parts of iodine per million parts of air-dry soil.

All reagents employed in the analysis were tested for iodine. The solution of $\text{Na}_2\text{S}_2\text{O}_3$ was checked frequently against iodine liberated in the above manner from a standard solution of KI .

RESULTS AND DISCUSSION

The iodine contents of the samples are given in table 1. There is no correlation between the iodine content of the soils examined and the prevalence or severity of cotton root rot. In this connection it is of interest to note that sample 110, taken from an area free of root rot, as judged by freedom from kill of growing cotton plants, is among the soils containing highest amounts of iodine, whereas sample 326, also relatively high in iodine, is from a badly infested area. There are wide variations in the amounts contained in the soils of the Houston and Wilson series, for which data are most abundant. The range and average iodine content of Houston black clay and Wilson clay loam of the Blackland prairie are given in table 2. The soils of the Houston series are definitely richer in iodine at all depths examined than are those of the Wilson series. In the Houston soils there was no relationship between the average iodine content and soil depth, but in the Wilson soils the iodine content tended to increase with soil depth to 36 inches.

Reference to table 1 will show that the heavy Irving clay soils examined contained generally more iodine than the lighter soils of the Wilson series, and are of about the same range in iodine content as the heavy Houston soils. The greatest amount in any sample analyzed was found in the one set of samples representing the Denton clay group. The smallest amounts of iodine, found in samples of Harlingen clay and Yahola silty clay loam of the Rio Grande Valley and Brazos River Valley, respectively, as compared to the amounts in soils of the Blackland, seem significant. The soils of the Brazos River Valley are of alluvial origin and are subject to periodic overflow and

rapid drainage. The iodine content of the soils from the Blackland section of Texas compares favorably with that reported on soils from some other states. The average iodine contents of all samples collected from this section at depths of 0-6, 6-12, 12-24, and 24-36 inches, were, respectively, 5.113, 5.625, 5.469, and 6.054 p.p.m. In six regions in South Carolina (5) the iodine in the surface 6 inches varied from 0.142 to 0.684 p.p.m., and in subsoil at a depth of 12 to 18 inches, from 0.377 to 1.181 p.p.m. Soils from six principal geological areas in Kentucky (4) are reported; the average content was 4.57, 4.11, 6.10, 4.07, 4.69, and 2.05 p.p.m. The amounts in soils from eight counties in Nebraska (1) ranged from none to 0.015 p.p.m.

The distribution of iodine in soils is frequently found to be correlated with the geological formations from which the soils have developed. McHargue and Young (4) found a higher iodine content in soils derived from limestone than in soils derived from sandstone strata. These investigators report that

TABLE 2
Range and average iodine content of Houston black clay and Wilson clay loam soils of the Blackland prairie of Texas
In p.p.m. air-dry soil

SOIL DEPTH <i>inches</i>	HOUSTON BLACK CLAY			WILSON CLAY LOAM		
	Minimum	Maximum	Average	Minimum	Maximum	Average
0-6	3.226	16.520	7.235	0.587	4.396	2.991
6-12	2.347	18.723	7.775	1.174	6.994	3.474
12-24	2.640	18.145	6.211	0.880	5.866	4.727
24-36	1.101	22.026	7.490	2.347	8.213	4.618

Orr and Leitch (7) found nearly twice as much iodine in fossiliferous limestones as in nonfossiliferous deposits. Mitchell (5) reports that soils from the middle section of South Carolina contain less iodine than those from the upper section and suggests a possible relationship with soil formation as the reason.

Also affecting the iodine content of a soil are such factors as soil texture, organic matter, and colloidal matter contents. Scharrer (8) in reporting on the iodine content of south German soils, concluded that in general a higher iodine content is associated with abundant colloids and organic matter. Nebraska (1) soils containing appreciable quantities of organic matter showed measurable amounts of iodine, whereas sandy soils low in organic matter did not show any iodine.

The relative abundance of iodine in soils from the Blackland section of Texas seems to be due primarily to geological formations of that area, individual differences in iodine content being influenced by such soil characters as texture, organic matter, and colloidal material, which affect retention of the iodine supplied by the soil parent material. These soils have developed

from soft calcareous parent material comprising deep beds of chalk, marl, or slightly limy clay. In general, they are well supplied with organic matter, are highly colloidal, and have a heavy texture. These characteristics are accentuated in soils of the Houston series and subordinated in those of the Wilson series.

SUMMARY

Some of the principal soil types of the Blackland prairie and surrounding sections of Texas were found to be relatively high in iodine.³ The iodine content of the soils examined apparently is not a factor influencing the present distribution of root rot of cotton, a disease prevalent in many of the soils of this section. The calcareous black clay soils examined generally contained more iodine than the lighter-textured noncalcareous Wilson clay loam soils. These are among the principal soil types of the Blackland and Grand Prairie sections of Texas.

The alluvial soils, Harlingen clay and Yahola silty clay loam, found extensively in the Lower Rio Grande Valley and Brazos Valley, respectively, contained less iodine than the residual soils of the Blackland prairie and Grand prairie sections.

The relationship between geological formations and the iodine content of soils is discussed, and the data presented are shown to be in harmony with some results reported by other investigators.

REFERENCES

- (1) ADOLPH, W. H., AND PROCHASKA, F. J. 1929 An iodine survey of Nebraska. *Jour. Amer. Med. Assoc.* 92: 2158-2160.
- (2) Association of Official Agricultural Chemists 1930 Official and Tentative Methods of Analysis, ed. 3. Washington, D. C.
- (3) CARTER, W. T. 1931 The soils of Texas. *Tex. Agr. Exp. Sta. Bul.* 431.
- (4) McHARGUE, G. S., AND YOUNG, D. W. 1933 The iodine content of the soil in Kentucky. *Soil Sci.* 35: 425-435.
- (5) MITCHELL, J. H. 1929 Study of iodine in South Carolina. *Science* (n.s.) 69: 650-651.
- (6) NORRIS, R. V., AND RAMA RAO, D. A. 1928 The estimation of iodine in soils. *Jour. Indian Inst. Sci.* 11A (pt. 7): 75-79.
- (7) ORR, J. B., AND LEITCH, I. 1929 Iodine in nutrition. *Med. Res. Council Rpt.* 123. London.
- (8) SCHARER, K. 1935 The iodine content of south German soils. *Ztschr. Pflanzenernähr., Düngung, u. Bodenk.* 39: 315-326. (Only abstract consulted.)
- (9) SHEAR, C. L. 1907 New species of fungi. *Bul. Torrey Bot. Club* 34: 305-317.

³ "An interesting report on the iodine content of city waters and vegetables in Texas," by G. S. Fraps and J. F. Fudge, of the Texas Agricultural Experiment Station, furnishes ample evidence that this element runs high in both. The report appears in *Food Res.* 4: 355-362, 1939. There has also appeared, since this manuscript was prepared, "Iodine in Texas Soils," by G. S. Fraps and J. F. Fudge, Texas Agricultural Experiment Station Bulletin 579, in which are given some additional analyses of the soils of the Blackland section of Texas.

PHYSICAL CHARACTERISTICS OF SOILS: VII. EFFECT OF IGNITION

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Heating soils to a high temperature is known to destroy their colloidal properties (1, 2, 3, 12). The exact temperature at which a change in base-exchange capacity may take place has been shown (2) to vary from 300 to 700°C., depending on the $\text{SiO}_2/\text{Al}_2\text{O}_3$ ratio. If we regard the soil as hydrated ferro-aluminosilicate, we can well imagine the change in its properties likely to be brought about by heating. It seems fairly certain that most of the outstanding properties of soil colloids must be associated with the presence of water of hydration, and the primary effect of heating lies in the removal of this water. A number of important properties like plasticity and cohesion, which may be due to this state of hydration, are destroyed completely when this water is driven out. The quantity of the basic material, i.e., the ferro-aluminosilicates, remains the same but may undergo progressive change with the increased temperature. A knowledge of these changes, especially in the acidoid properties of the soil, is of interest in elucidating the fundamental properties of soil colloids.

In order to avoid complications, the soils used for this study, unless otherwise mentioned, were acid treated to remove the exchangeable bases. The following properties were studied before and after heating the soils to various temperatures: titration curves, base-exchange capacity, reaction with ammonia, production of free alkali, dispersion (clay content), destruction of humus and decomposition of calcium carbonate, loss of weight on ignition (dehydration), moisture absorption, puzzolonic properties, sticky point and rolling limit, aggregate analysis.

TITRATION CURVES

The acidoid property of the soil is revealed through its titration curve, which gives the alkali equivalent, i.e., $T/2$ value (quantity factor) or pK value (intensity factor). A black cotton soil containing 56 per cent clay was used for this study, after being freed from exchangeable bases by exhaustive treatment with 0.05 *N* HCl. The H-soil thus produced was heated to different temperatures for 6 hours in an electric muffle furnace. Titration curves with NaOH were determined with the glass electrode, and $T/2$ and pK values were found from the titration curves as explained previously (7). The titration

curves are shown in figure 1, and the pK values are given in table 1. It will be seen that the $T/2$ value is hardly affected by ignition, though the values show rather large variations because of the difficulty of interpolation in the flat portions of the titration curves. The pK values show a progressive increase with heat. Ignition, therefore, has the effect of making the soil acidoid weaker, its total quantity remaining virtually the same.

BASE-EXCHANGE CAPACITY

Base-exchange capacity has been used so widely in all studies relating to the effect of heat on the colloidal properties of soils that it seemed necessary to include it in this investigation in spite of the vagueness of the meaning attached to this term and the unsatisfactory nature of the methods of estimating it (11). The potassium chloride-ammonium carbonate method was used for the purpose (5). The method consists in leaching the soil with N KCl and

TABLE 1
Effect of ignition on various properties of P.C. 13 A.T. soil

TEMPERATURE	$T/2$	pK	BASE-EXCHANGE CAPACITY		NH ₃ ABSORPTION	CLAY CONTENT
			Without KOH	With KOH		
°C.	<i>m.e.</i>		<i>m.e.</i>	<i>m.e.</i>	<i>m.e.</i>	<i>per cent</i>
95	48.00	5.07	29.0	47.9	64.4	43.3
225	39.00	5.36	29.0	48.1	64.1	38.0
300	45.25	5.62	30.5	48.4	59.9	28.5
400	41.40	6.11	28.0	49.1	52.1	6.3
520	35.25	6.20	21.6	44.6	51.0	6.3
610	41.75	6.98	17.9	41.8	49.7	6.3
710	50.00	7.54	11.5	33.9	33.8	6.3
800	39.1	7.84
910	52.00	8.69

displacing with $(\text{NH}_4)_2\text{CO}_3$ the potassium taken up. A slight modification of the method was made by adding to the soil in the first instance sufficient potassium hydroxide to raise the pH value to 8.5. Since the initial pH values of the ignited soils were different, this modification brought them to a uniform initial condition. Base-exchange capacity was measured with both the methods. The results are included in table 1. It is seen that base-exchange capacity is constant up to 400°C. Ignition above this temperature leads to a progressive decrease, which is consistent with other changes that seem to take place when the soil is heated at higher temperatures. The reason for higher base-exchange capacity when the soil is subjected to a preliminary treatment with KOH, as compared with untreated soil, have been discussed in a previous paper (11).

REACTION WITH AMMONIA

Interaction between ammonia and soils has been discussed in a previous publication (8). It was shown that ammonia reacts with soil acidoid just

like other bases and therefore it could be used for measuring the base equivalent of an acidoid neutralized to a certain pH value. The treatment consists in adding excess of ammonia solution to a H-soil, boiling to remove the excess, and estimating, by distillation with lime, the ammonia taken up. The results of this study, given in table 1, show that the amount of ammonia reacting with the soil decreases with the increase of temperature of ignition. There is a point of inflection between 300 and 500°C.

TABLE 2
Production of free alkali on ignition

0.1N ALKALI PRESENT IN 10 GM. SOIL	pH VALUE		ALKALI SET FREE
	Before ignition	After ignition	
cc.			cc.
<i>Na saloid</i>			
0.0	3.82	7.73	0.2
2.5	4.24	7.75	0.5
7.5	4.90	7.65	0.3
15.0	5.73	7.58	0.0
17.5	6.00	7.70	0.4
25.0	7.70	7.70	0.2
40.0	9.80	9.41	0.2
60.0	10.95	9.93	0.4
<i>Ca saloid</i>			
5.0	3.75	7.80	0.2
12.5	4.52	8.25	0.0
17.5	6.12	7.85	0.4
25.0	7.70	8.11	0.2
40.0	9.05	8.99	1.8
60.0	9.97	10.12	2.0

PRODUCTION OF FREE ALKALI

We have seen that the acidoid properties of soil colloids are partly destroyed on heating. It appeared logical to conclude from this that if the soil acidoid was neutralized with a base and then heated, part or all of the base might be released. This supposition appeared to offer attractive possibilities of determining the exchangeable bases by ignition in soils free from CaCO_3 . Sodium soil obtained by neutralizing a H-soil to various pH values was ignited, and the amount of alkali set free was determined. The results, given in table 2, show that hardly any alkali is set free. Some alkali, apparently set free when 40 and 60 cc. of 0.1 N alkali are added to 10 gm. of soil, would be leached out of the soil even without ignition.

The results are very important, as they appear to give us an insight into the real mechanism of the destruction of colloidal property of soil on ignition. If ignition merely resulted in dehydration and destruction of the acidoid prop-

erties of soil colloids, then alkali would necessarily be set free. If, on the other hand, ignition leads to a fusion of the colloidal particles and the production of larger particles, then no alkali will be set free. The latter view is confirmed by the fact that if a soil containing exchangeable sodium or calcium is ignited, the whole of the base becomes fixed in the nonreplaceable form and cannot be displaced by any of the usual replacing agents. The progressive increase in the pH value of a H-soil on heating also leads to the same conclusion. It must be understood that a soil on drying can form water-stable aggregates, but these aggregates can always be resolved into individual particles by mechanical or chemical methods of dispersion. Ignition, on the other hand, leads to the formation of permanent aggregates that cannot be resolved by physico-chemical treatments into smaller particles originally present. These aggregates are in all respects similar to larger particles and correspond in properties to silt fractions in natural soils.

DISPERSION (CLAY CONTENT)

The percentage of conventional clay (0.002 mm. diameter) has an important bearing on the colloidal properties of soils, and the effect of heating on this factor is of interest. Clay was determined by shaking for 24 hours with sufficient NaOH to raise the pH value to 10.8 (9). The results, included in table 1, show the progressive decrease in the clay content, which reaches a minimum value at 400°C. It seems that the dehydration of clay at higher temperatures leads to a progressive fusion of clay particles, resulting in stable aggregates which cannot be disintegrated by ordinary methods of dispersion and therefore cannot be distinguished from coarser fractions.

Complete mechanical analyses of the heated soils were made by the simple method of shaking with NaOH to pH 10.8. The summation curves are shown in figure 2. It will be seen that except for a minor shift, the curves continue to be similar until the temperature is raised above 400°C., which may be regarded as the fusion temperature of colloidal particles. At this temperature, there is an abrupt shift in the curve as a whole, and further heating again causes only a minor change. The remarkable similarity of all the curves is noteworthy.

DESTRUCTION OF HUMUS AND DECOMPOSITION OF CaCO_3

Destruction of humus and the decomposition of CaCO_3 are two of the most important changes intimately associated with the ignition of soils, though not directly concerned with its colloidal properties. The total loss of weight accompanying the ignition of soil is made up of the following factors: dehydration, destruction of the organic matter or humus, and decomposition of calcium carbonate.

In order to determine the magnitude of each factor, a number of soils were heated to different temperatures in the natural state, after acid treatment (destruction of CaCO_3), and after destruction of humus by alkaline potassium

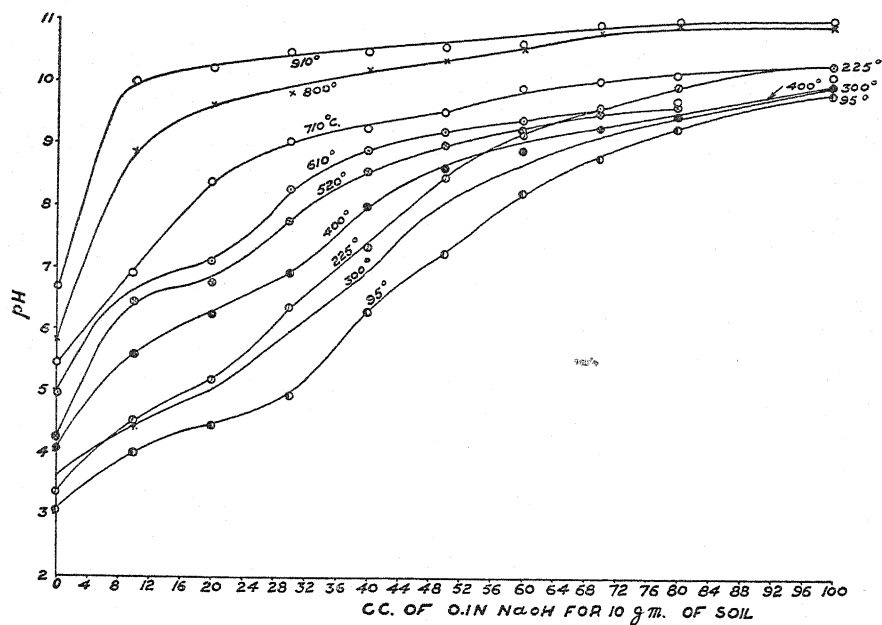


FIG. 1. TITRATION CURVES OF P.C. 13 A.T. SOIL HEATED TO VARIOUS TEMPERATURES

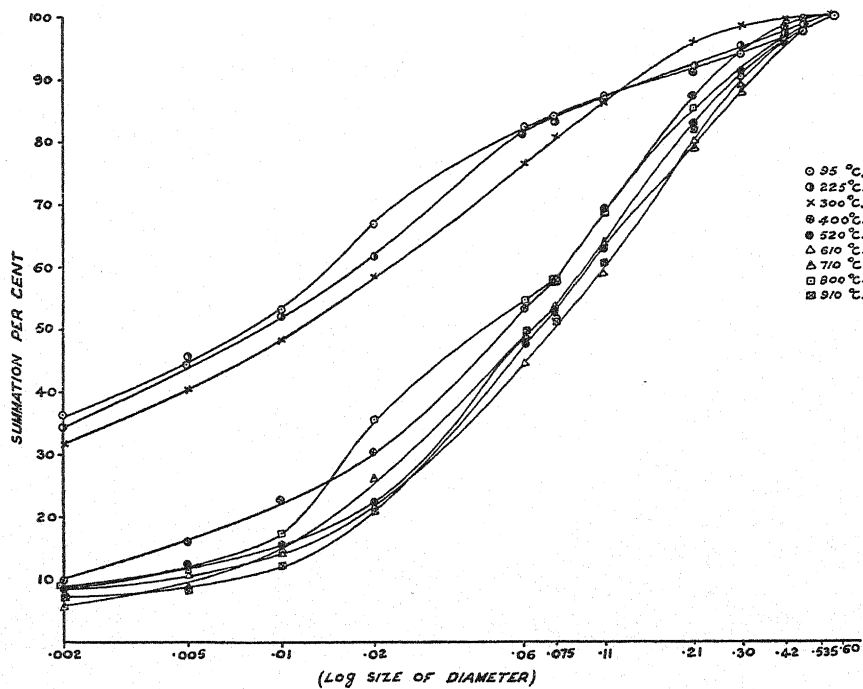


FIG. 2. SUMMATION CURVES OF P.C. 13 A.T. SOIL HEATED TO VARIOUS TEMPERATURES

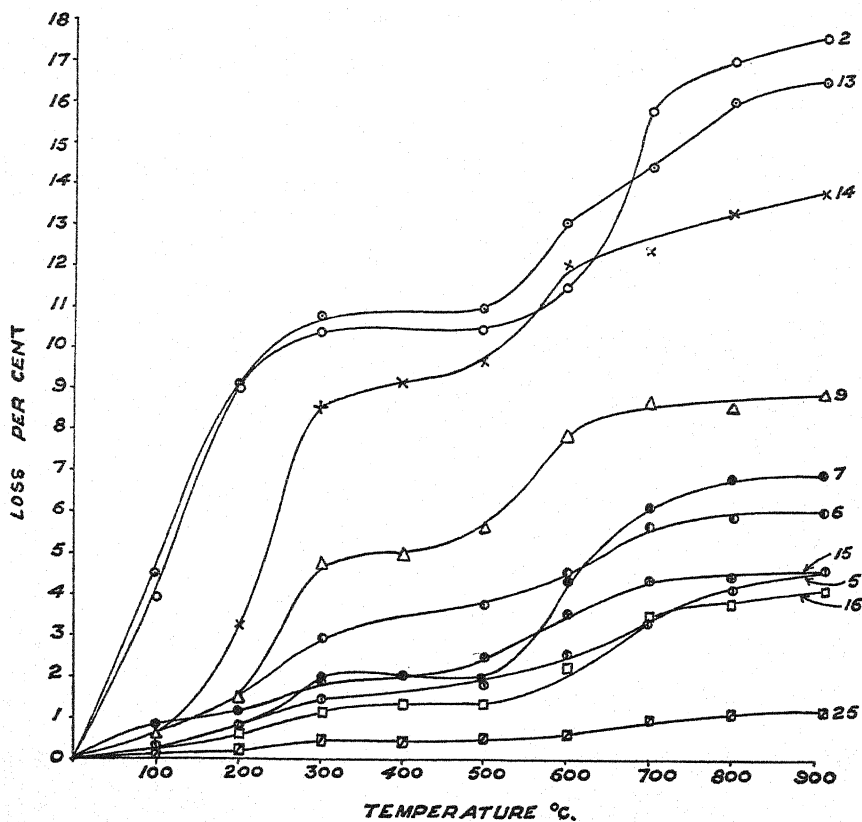


FIG. 3. LOSS OF WEIGHT ON IGNITION OF NATURAL SOILS

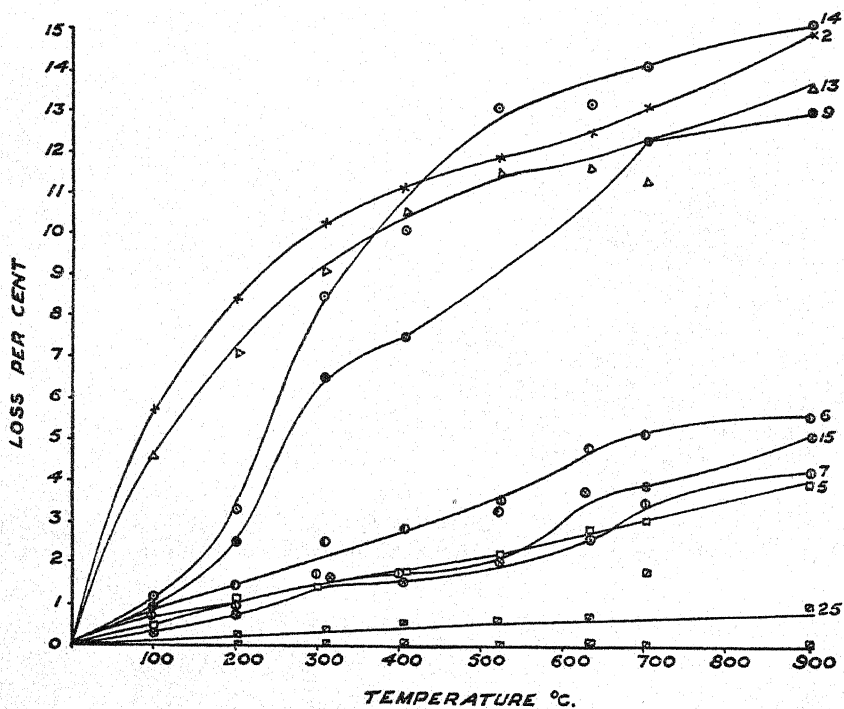


FIG. 4. LOSS OF WEIGHT ON IGNITION OF ACID-TREATED SOILS

permanganate (10). The progressive loss of weight against the temperature of heating is shown in figures 3, 4, and 5.

The curves for various soils show a remarkable similarity. The curve for the destruction of calcium carbonate alone is also shown in figure 5. Humus is completely burnt up at 400°C. The major portion of the loss in soils is confined to temperatures below 500°C.; CaCO_3 , on the other hand, is hardly decomposed at this temperature, and at 600°C. its decomposition is practically complete.

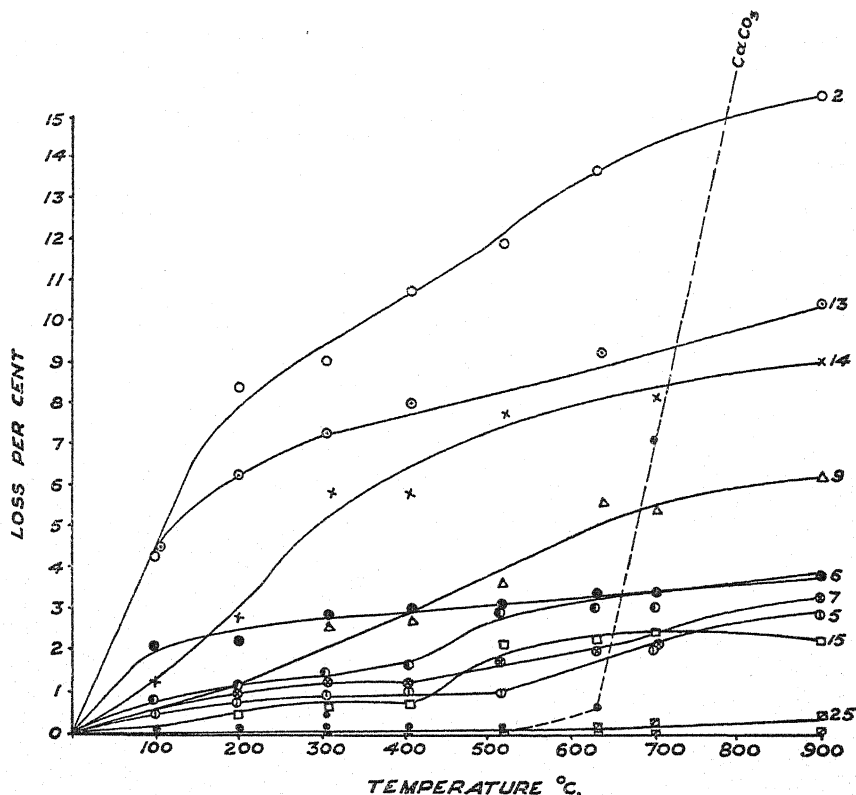


FIG. 5. LOSS OF WEIGHT ON IGNITION OF HUMUS-FREE SOILS

MOISTURE ABSORPTION

Absorption of atmospheric moisture is a characteristic property of soil colloids which is bound to be affected by heat. The absorption of moisture at various humidities by a soil heated to different temperatures is shown in figure 6. A progressive decrease in hygroscopicity with increasing temperatures is apparent at all humidities.

It was shown in a previous publication that the absorption of moisture between 10 and 70 per cent humidity is correlated with the clay content of soils

(4). It is of interest to see whether the decrease in clay content due to dehydration is correlated with the decrease in hygroscopicity. The relation be-

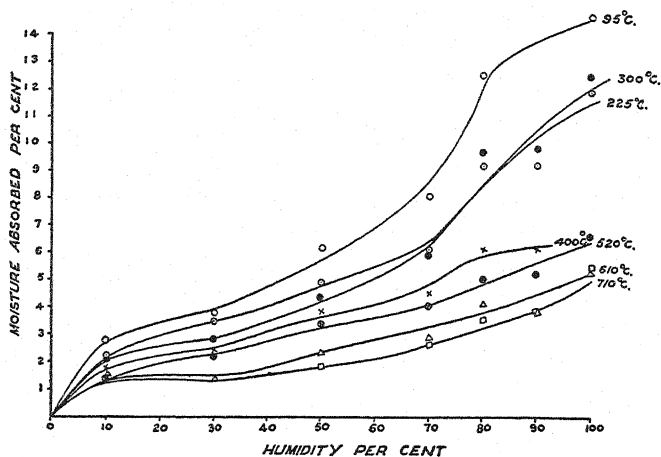


FIG. 6. MOISTURE ABSORPTION AT VARIOUS HUMIDITIES BY SOIL HEATED TO VARIOUS TEMPERATURES

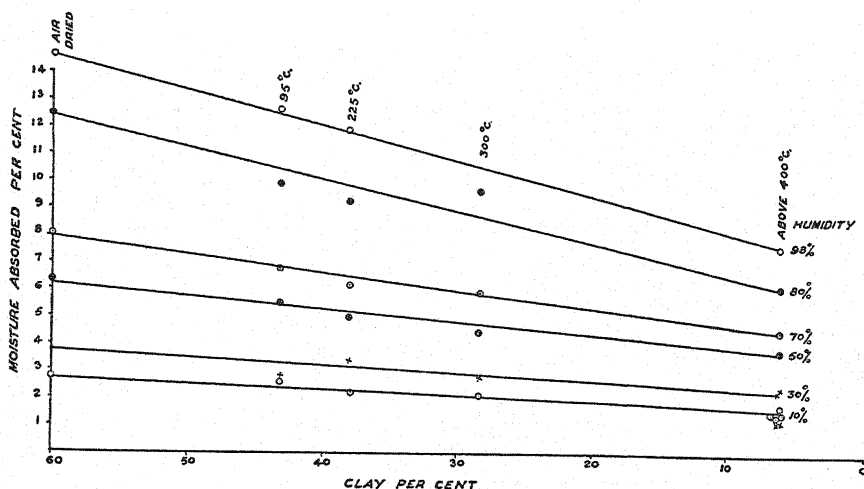


FIG. 7. RELATION BETWEEN CLAY AND HYGROSCOPICITY OF SOIL HEATED TO VARIOUS TEMPERATURES

tween clay and moisture absorption at various humidities is shown in figure 7. It will be seen that the moisture absorption decreases with the decrease in the clay content, which diminishes as the temperature of ignition increases.

PUZZOLONIC PROPERTIES

Methods of determining soil cohesion have been discussed in a previous publication (6). As the soil, when heated, loses its plasticity, it develops puzzolonic property, by virtue of which it can combine with lime and set under water like hydraulic cement. To determine at what temperature this property comes into prominence, a common Punjab soil was heated to different temperatures for 4 hours, mixed with 20 per cent lime, and kept in molds under wet sand for 24 hours, after which the semispherical pellets were kept under water for 7 days.¹ The cohesion was then tested. The results, in table 3, show that the higher the ignition temperature, the better the develop-

TABLE 3
Cohesion of soil heated to different temperatures and mixed with 20 per cent lime

TEMPERATURE	COHESION
° C.	kgm.
120	4.5
300	6.7
500	14.1
700	15.5
920	20.0

TABLE 4
Increase in cohesion of ignited soil pellets on being kept in lime water

TIME KEPT IN LIME WATER	COHESION
days	kgm.
0	31.0
7	42.5
14	40.0
21	42.1
28	44.1

ment of the puzzolonic property. It is interesting to note that the development of the puzzolonic property follows exactly the destruction of the colloidal properties (cf. table 3 and table 1, column 5). The increase in the cohesion of ignited soil pellets as a result of contact with lime is shown in table 4. It will be seen that cohesion increases appreciably when the pellet is kept in lime water for 7 days; longer contact has no apparent effect.

ROLLING LIMIT AND STICKY POINT

Rolling limit is defined as the moisture percentage at which a soil can be rolled to give a thread 1 inch long and $\frac{1}{8}$ inch thick without breaking. Sticky point is the moisture percentage at which the soil begins to stick to the hand.

¹ This technic is similar to that used in cement testing.

These are useful properties which can be expressed as single values for characterizing soils. It was found that these properties are only slightly affected by heating the soil up to 400°C. Above this temperature, the determination of both the rolling limit and the sticky point abruptly becomes indefinite: the soil loses its plasticity and behaves like sand or coarse silt.

SUMMARY

The effect of heating soil to various temperatures on its physicochemical properties was studied.

The progressive decrease in the colloidal properties is most likely due to the fusion of the smaller particles, resulting in stable aggregates which cannot be disintegrated by ordinary methods of dispersion.

REFERENCES

- (1) BROWN, G. H., AND MONTGOMERY, E. T. 1913 Dehydration of clays. U. S. Dept. Com. Bur. Standards Technol. Paper 2.
- (2) KAMOSHITA, Y. 1937. On the effect of heating soil upon the base exchange capacity of soils. *Jour. Sci. Soil Japan* 2: 343.
- (3) KELLEY, W. P., DORE, W. H., AND BROWN, S. M. 1931 The nature of the base-exchange material of bentonite, soils, and zeolites, as revealed by chemical investigations and X-ray analysis. *Soil Sci.* 31: 25-55.
- (4) PURI, A. N. 1932 A new method of determining clay content of soils by moisture absorption at 70 per cent humidity. *Soil Sci.* 33: 405-411.
- (5) PURI, A. N. 1934 A new method of determining base exchange capacity of soils. *Soil Sci.* 37: 105-108.
- (6) PURI, A. N. 1937 Physical characteristics of soils: I. New methods of measurements. *Soil Sci.* 44: 481-487.
- (7) PURI, A. N., AND ASGHAR, A. G. 1937 Titration curves and dissociation constants of soil acidoids. *Soil Sci.* 45: 359-367.
- (8) PURI, A. N., AND ASGHAR, A. G. 1938 Reaction between ammonia and soils. *Soil Sci.* 45: 477-481.
- (9) PURI, A. N., AND LAL, M. 1938 Dispersion and stability of soil colloids in water: I. Auto-disintegration. *Punjab Irrig. Res. Inst. Res. Pub.* Vol. 4.
- (10) PURI, A. N., AND SARUP, A. 1937 The destruction of organic matter in the preliminary treatment of soils for mechanical analysis. *Soil Sci.* 44: 87-89.
- (11) PURI, A. N., AND UPPAL, H. L. 1939 Base exchange in soils: I. A critical examination of the methods of finding base-exchange capacity of soils. *Soil Sci.* 47: 245-253.
- (12) STEENKAMP, J. L. 1928 The effect of dehydration of soils upon their colloid constituents. *Soil Sci.* 25: 163-182.

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BIOLOGY

CONTROL OF FECAL-BORNE DISEASES IN NORTH CHINA: XI. CHEMICAL NATURE OF SHANTUNG FARM MANURE¹

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For many years agricultural workers in the West have been intensely interested in the common practices developed in China and Japan for the return of wastes to the soil as fertilizers. Very few quantitative studies, however, have yet been published which elucidate, in a critical manner, the actual workings of Oriental fertilizer systems. The classical observations of King (7) still constitute one of the chief sources of information concerning Chinese agriculture, even though they were made 30 years ago. The recent publications of Buck (3, 4) have brought together an immense amount of information about Chinese agriculture and have painted the broad outlines of the operation of the system throughout the land, yet specific studies of the production and use of fertilizers are conspicuously lacking.

In any discussion of agriculture in China it must be kept clearly in mind that the practices followed in different parts of the country vary widely. Buck (4) has divided China into two great agricultural regions and eight areas. Each of these areas differs in certain important respects from the others. Methods of handling and using wastes for fertilizers tend to vary in the different areas. The practices described in this paper refer specifically to conditions in West Shantung, but they are fairly commonly found throughout the winter-wheat-kaoliang area, which comprises all of Shantung, most of Hopei, and parts of Honan, Anhui, and Kiangsu Provinces. This is the most populous of the eight areas described by Buck, having almost twice the population of any other single area and nearly one third of the rural population of the entire country.

This paper is one of a series being published in various agricultural, medical, and biological journals, which report the results of an extended study of the problems involved in developing a system of agricultural sanitation for use in North China. This program attempts to give due weight to the demands of both agriculture and public health, and seeks a solution of the problem of sani-

¹ This is the eleventh of a series of papers reporting the results of cooperative studies on agricultural sanitation carried on by the Cheeloo University Department of Biology under the direction of Gerald F. Winfield and by the Yenching University Department of Chemistry under the direction of Stanley D. Wilson. These studies are financed by the Rockefeller Foundation.

tation by agricultural means so that to the values of permanent agriculture as practiced in China may be added the advantages of modern sanitation.

MATERIALS AND METHODS

The data presented in this paper were derived from the study of the farm manure produced on 32 farms in West Shantung.² The methods in vogue for handling farm manure throughout a large part of this area are so different from those used in other parts of China and in other countries that a detailed description is necessary, although a similar account has been published elsewhere (14).

The fertilizer produced on the farm is accumulated and stored in a pit which serves the triple purpose of compost pit, pigpen, and latrine. It varies in size and shape but usually is 8 to 15 feet square by 4 to 6 feet deep. The capacity of the pits of the 32 farms studied varied from 14 to 1008 cubic feet (table 1). At one side of this pit there is usually a shed, in many instances large enough to shelter a cow or a horse. The door of this shed opens on the main courtyard around which the other farm buildings are placed. The shed serves the double purpose of a shelter for the animals and a place where the family may defecate, although in some cases a separate stable is maintained. The pit is usually lined with brick or stone, and the bottom is made by tamping a mixture of lime and clay into a hard-packed mass that will hold water. There are steps that permit the pig, when present, either to rest on the ground or to wallow in the muck in the pit. The pig gains part of its subsistence by eating the freshly passed stools, which are high in unused carbohydrates, and his activity serves to keep the materials in the pit stirred. All animal manure from the other farm animals is placed in the pit along with the field earth which is used for bedding. No straw bedding is employed because of the necessity of using such plant material for fodder or fuel. The total amount of soil added is, in many instances, so large that the term "soil compost" seems an appropriate designation for farm manure prepared in this way. In addition to human and animal manure, all household wastes and such manure as the farmer or his son may be able to pick up on the cart roads, together with the ashes from the stove, are added to the compost pit. Water is added during dry weather, and during the rainy season the pit usually contains several inches or even several feet of water. In many cases the compost is removed only once a year, although on some farms it may be removed two or more times. Almost all pits are cleaned in early spring. The soil compost is first carried to the village street or to the threshing floor, where it may be partly dried and pulverized by being turned several times. It is then carted to the fields, placed in small piles, and further dried. Much of it is completely dry when it is finally spread over the field or along the rows of growing winter wheat. All told, the Shantung farmer expends a great deal of time and energy collecting, preparing,

² We wish to acknowledge the assistance of James C. Scott in the analysis of data.

TABLE 1
Materials used in the production of soil compost manure on 32 Shantung farms

FARM NUMBER	PERIOD OF ACCUMULATION		MONTHS EACH KIND OF ANIMAL KEPT DURING PERIOD OF ACCUMULATION				PERSONS USING LATRINE		SOIL ADDED PER DAY	OTHER MATERIALS ADDED	CAPACITY OF PIT	AREA OF LAND FERTILIZED	CROP
	mos.		Cow	Donkey	Mule	Pig	Adults	Children					
									chin*				
Lungshan area													
1	12	0	0	12	12	2	2	30	None	700	6.5		
2	12	0	12	12	0	3	3	70	None	315	7.5		
3	12	0	12	0	0	2	1	30	Refuse	384	6.0		
4	12	0	0	0	6	2	0	20	Refuse	293	2.5		
5	12	0	8	0	0	4	1	20	None	240	5.0		
6	12	6	0	0	0	3	5	30	Refuse	525	3.75		
7	6.5	12	0	0	24	5	2	100	Feces	1008	12.0		
8	12	12	0	0	12	5	1	50	Refuse	378	8.7		
9	12	12	12	0	12	2	4	100	Refuse	384	10.0		
10	12	12	0	0	0	3	0	30	Refuse	210	4.0		
11	6	0	0	0	0	5	3	0	Manure & feces	180	2.5		
12	7	12	0	0	24	4	1	100	None	384	9.0		
13	12	12	0	0	12	4	1	60	Refuse	756	10.0		
14	12	12	0	0	0	4	2	30	Refuse	432	3.6		
15	12	0	10	0	0	4	2	30	Refuse	720	9.0		
16	12	0	9	0	0	5	0	8	Refuse	320	4.5		
Tsinan area													
17	4	4	0	0	0	0	0	80	None	230	2.0	Millet	
18	5	4	0	0	0	2	6	30	Ashes	172	5.0	Wheat	
19	3.5	1.5	0	2	4	0	0	100	Refuse	231	5.0	Millet	
20	4	0	0	0	12	0	0	130	None	210	6.0	Wheat	
21	6	0	0	0	0	2	3	80	Manure, feces, & refuse	14	1.0	Wheat	
22	12	0	0	0	0	5	0	Pond mud	Manure, feces, & refuse	180	4.0	Vegetables	
23	4	0	0	0	0	0	0	0	Feces	70	0.7	Vegetables	
24	10	0	0	0	0	2	0	0	Manure, & feces cakes	216	2.0	Vegetables	
25	18	0	0	0	72	5	3	0	Refuse	319	5.0	Millet	
26	7.5	7	7	0	0	7	9	0	Refuse	88	3.0	Millet	
27	3	0	0	1.5	18	0	0	120	None	385	10.0	Millet	
28	2.5	0	0	0	0	6	0	0	Ashes & refuse	61	3.5	Wheat	
29	6	0	0	0	0	1	0	0	Coal ashes & refuse	202	4.0	Millet	
30	4	0	0	0	16	3	3	120	Manure	240	3.0	Millet	
31	2.5	2.5	0	0	0	3	0	10	Refuse	141	3.0	Millet	
32	7	0	0	0	0	4	3	0	Coal ashes & refuse	239	4.0	Wheat	

* The chin is equal to 500 gm.

† The mu is equal to 0.1647 acre.

and using this soil compost. If he stores it outside his pit for any considerable time he is very likely to cover it with a layer of straw-reinforced mud to protect it from rain and theft.

Of the 32 farms studied, 16 were situated in a group of villages in the Lungshan market town area about 20 miles east of Tsinan. An investigator and a work crew were sent to each farm in March 1937, when pits were being emptied, to weigh and sample the entire soil compost output. A survey schedule covering essential information, much of which is presented in table 1, was filled in for each farm. The sample was taken by quartering down a stack made of every fifth basketful weighed. The samples were brought to the laboratory, the moisture loss on air drying was determined, and the air-dry material was ground for analysis. The analytical methods followed were standard ones as laid down by the A. O. A. C. (1) except in certain cases as indicated in the text below.

The second 16 samples were procured by the same means during the early spring of 1939 from farms near Tsinan city. A number of these had their farmsteads inside the east suburb wall with their fields outside. Methods of preparing and using fertilizers, however, closely follow those current in rural areas. The samples were handled somewhat differently from the first series. The wet material was ground in a food chopper, and pH, moisture, Kjeldahl nitrogen, and ammoniacal nitrogen (by MgO distillation) were determined on the fresh wet material. The other determinations were made on air-dry material.

The units of weight and measure used in this paper conform to the official standards set up by the Chinese Government. They are the *chin*, which is equivalent to 500 gm., and the *shih mu*, which is equal to 0.1647 acre.

PRESENTATION OF DATA

Materials forming the soil compost manure

The soil composts studied were prepared from varying combinations of raw materials, as shown in table 1. It will be noted (column 2) that most of the pits in the Lungshan area had not had the compost removed during the preceding year, whereas most of those in the Tsinan area had been cleaned 2.5 to 7 months previously. In the succeeding four columns are shown the total numbers of animal-months that the four most common farm animals were kept during the period of fertilizer accumulation under study. The manure produced by these animals and passed in the stable or near the house may be assumed to have been placed in the pit. It is of interest to note that 13 of the 32 farms kept cows during at least some part of the period under study, 12 kept pigs, 7 kept donkeys, and 4 kept mules. One of the Lungshan and seven of the Tsinan farms kept no animals at all. From 2 to 8 persons used the pen-latrine for defecation in the Lungshan farms, whereas 4 of the Tsinan farms reported that the pits were not used as latrines. Observations made on

rural families (14) would indicate that a fairly large percentage of the fecal material of the children does not reach the compost pit but is scattered promiscuously about the village. The amounts of field soil added are rather large (column 9). One farm put large quantities of pond mud in the pen-latrine, and eight added no soil to their compost. Only seven farms reported that no additional material from outside, such as feces or manure collected from the roads, refuse, or even coal ashes, was added. It is likely that these farms added some other materials but not regularly enough to deem it worth reporting. In at least some of the poor families such manure collected from the outside may be the major source of fertilizer. The fertilizer studied was applied to 0.7 to 12 mu (0.12 to 1.98 acres) of land. The crops fertilized were

TABLE 2

Chemical analysis, in per cent, of 32 Shantung farm manures, and fertilizer production, in chin, on 32 farms calculated to a 12-month basis*

	CHEMICAL ANALYSIS, MOISTURE-FREE BASIS				FERTILIZER PRODUCTION			
	Maximum	Minimum	Mean	Standard deviation of mean	Maximum	Minimum	Mean	Standard deviation of mean
Total wet weight	167,000	4,000	31,600	3,500
Organic and ammoniacal nitrogen	1.35	0.17	0.43	0.062	453.0	18.0	80.3	14.10
Nitrate nitrogen	0.066	0.0	0.015	0.014	18.0	0.0	2.82	0.75
Total organic and inorganic nitrogen	1.355	0.196	0.45	0.042	453.0	20.5	83.1	13.7
Total carbon	12.28	2.59	5.69	0.42
Carbonate carbon	2.01	0.09	0.64	0.08	476.0	19.0	127.8	21.7
Organic carbon	11.22	2.50	5.05	0.39	3431.0	253.0	916.6	112.3
Phosphorus, P_2O_5	1.21	0.16	0.47	0.041	310.0	22.0	80.4	10.1
Potassium, K_2O	1.45	0.67	0.87	0.027	1036.0	29.0	192.8	33.4

* The chin is equal to 500 gm.

wheat, harvested soon afterward and followed by beans and corn in most cases; millet, which is one of the most important summer crops of the region; and vegetables.

Annual fertilizer production per farm

In order to make the amounts and the fertilizer constituents of soil compost comparable from farm to farm, all results were calculated to a 12-month basis and are summarized in table 2.

The total wet weight produced annually per farm was very variable, ranging from 4,000 to 167,000, mean 31,600, chin for the 32 farms (table 2). This represents an average total annual production of about 15 metric tons of soil compost per farm. The percentage of dry matter ranged from 56.6 to 89.7, average 70.2, for the entire series of 32 farms.

Nitrogen in manure

The concentration of total organic and ammoniacal nitrogen, as determined by the Gunning method, in the manures (table 2) ranged from 0.17 to 1.35, mean 0.43, per cent. It should be remembered that the nitrogen in the Lungshan series was determined on air-dried samples, whereas that in the Tsinan series was determined on wet samples. The production per farm of organic and ammoniacal nitrogen, calculated on a 12-month basis for the 32 farms, ranged from 18 to 453 chin, with a mean of 80.3 σ 14.1 chin. It should be

TABLE 3

Losses of nitrogen from Shantung farm manure, Tsinan area, after air drying of the sample
All data in per cent on a moisture-free basis

SAMPLE NUMBER	WET SAMPLE DETERMINATIONS			AIR-DRY SAMPLE	WET - DRY SAMPLE DIFFERENCE	PERCENTAGE LOST ON AIR DRYING
	Total nitrogen	NH ₃ -N	NH ₃ -N, per- centage of total nitrogen	Total nitrogen		
732	0.42	0.08	19.00	0.35	0.07	16.70
733	0.58	0.11	19.00	0.52	0.06	10.30
734	0.41	0.13	31.70	0.30	0.11	26.80
735	0.37	0.11	29.70	0.34	0.03	8.10
736	0.55	0.11	20.00	0.51	0.04	7.30
737	0.62	0.09	14.50	0.63	+0.01	+1.60
738	1.35	0.28	20.70	0.95	0.40	29.60
739	0.98	0.17	17.40	0.95	0.03	3.10
746	0.49	0.19	38.80	0.40	0.09	18.40
747	0.62	0.19	30.70	0.44	0.18	29.00
748	0.38	0.14	36.80	0.32	0.06	15.80
749	0.67	0.22	32.80	0.54	0.13	19.40
750	0.38	0.13	34.20	0.31	0.07	18.40
751	0.66	0.11	16.70	0.47	0.19	28.80
752	0.54	0.13	24.10	0.44	0.10	18.50
753	0.36	0.10	27.80	0.29	0.07	19.40
Mean.....	0.59	0.14	25.90	0.49	0.101	16.80
Sigma.....	0.064	0.0013	1.91	0.051	0.024	2.34

kept in mind that the large amount of field soil added to the composts contained an estimated average of 10 chin per farm.

The amount of ammoniacal nitrogen was determined on the Tsinan series of wet samples by direct MgO distillation (table 3). The mean percentage was 0.14 σ 0.0013 calculated on a moisture-free basis. This represented from 14.5 to 38.8, mean 25.9 σ 1.91, per cent of the total nitrogen.

Nitrate nitrogen was determined by the phenoldisulfonic acid method. It was present in measurable quantity in all but one of the Lungshan series and in 11 of the 16 Tsinan. The lack of nitrate in the five Tsinan farm composts

was likely due to the short period of accumulation, since only one of the five pits contained material as old as 7 months, the storage period for the other four ranging from 2.5 to 4 months. The nitrate present ranged from none to 0.066 per cent with a mean of $0.015 \sigma 0.014$ per cent for the 32 farms (table 2). The production of nitrate nitrogen per farm calculated to a 12-month basis ranged from none to 18.0 and averaged $2.82 \sigma 0.75$ chin.

The figures for total organic and inorganic nitrogen in terms of percentage concentration and yearly production are shown in table 2. The concentration ranged from 0.196 to 1.355, mean $0.45 \sigma 0.042$, per cent, and the production ranged from 20.5 to 453.0, mean $83.1 \sigma 13.7$, chin per farm. As has already been pointed out, the farmers usually dry their fertilizer before plowing it in. They do this to get a more even distribution of material over the field and also, they say, to kill insect larvae and eggs. On the basis of analyses made on the Tsinan series (table 3), we found that $16.8 \sigma 2.34$ per cent of all the nitrogen present was lost when the samples were air dried. This represents about 65 per cent of the ammoniacal nitrogen present. In view of the results shown by field test reported in the literature (6) on the effects of drying on yield, it would seem likely that the present practice of drying fertilizer is not a good one.

Carbon in manure

The total percentage of carbon as determined by wet combustion (table 2) ranged from 2.59 to 12.28, average $5.69 \sigma 0.42$. The carbonate carbon as determined by a Collins calcimeter ranged from 0.09 to 2.01, mean $0.64 \sigma 0.08$, per cent for the 32 farms; in other words, 11.24 per cent of the total carbon present was carbonate carbon. When the carbonate carbon was subtracted, the organic carbon ranged from 2.50 to 11.22, mean $5.05 \sigma 0.39$, per cent.

When the percentage of organic matter for the Tsinan series was calculated by multiplying the organic carbon by 1.724, it was found (table 4, II) to range from 4.45 to 19.34, mean $10.72 \sigma 1.03$. The organic matter present in these Shantung farm manures, therefore, does not exceed in amount that present in rich mineral soils. Because of the dilution with soil, none of these manures approach the organic matter content of farmyard manures as prepared in the west.

It is of interest in this connection to compare three other methods of calculating total organic matter with that based on organic carbon content. The first of these is loss on ignition with carbonate carbon subtracted (table 4, I). The mean loss on ignition was $15.79 \sigma 1.31$ per cent. This figure is significantly higher than that based on organic carbon ($10.72 \sigma 1.03$). When the percentage of organic matter was calculated by multiplying the total Kjeldahl nitrogen by 20, the mean was $11.73 \sigma 1.29$ per cent. When $\text{NH}_3\text{-N}$ was subtracted before multiplying, the mean was $8.86 \sigma 1.12$ per cent. These last two means are not significantly different from each other or from the mean based on organic carbon.

Carbon-nitrogen ratio

When the carbon-nitrogen ratios of the Tsinan series of samples were calculated by the use of organic carbon ($\text{CO}_2\text{-C}$ subtracted) over organic nitrogen ($\text{NH}_3\text{-N}$ subtracted), they were found to range from 6.97 to 16.23 with a mean of 10.41. These C/N ratios indicate that the organic matter present is approaching a composition which is typical of soil humus.

TABLE 4
Comparisons of percentages of organic matter calculated by four different methods for 16 Shantung farm manures from Tsinan*

SAMPLE NUMBER	I	II	III	IV
732	16.20	11.8	6.8	8.4
733	17.00	12.9	9.4	11.6
734	12.10	7.9	5.6	8.2
735	8.60	4.5	5.2	7.4
736	15.80	11.7	8.8	11.0
737	15.10	9.3	10.6	12.4
738	22.30	17.7	21.4	27.0
739	28.00	19.3	16.2	19.6
746	10.80	7.3	6.0	9.8
747	20.60	14.7	8.6	12.4
748	8.60	5.0	4.8	7.6
749	14.10	10.2	9.0	13.4
750	13.10	7.5	5.0	17.6
751	20.10	11.8	11.0	13.2
752	18.50	12.0	8.2	10.8
753	11.50	7.8	5.2	7.2
Mean.....	15.78	10.72	8.86	11.73
Standard deviation of mean.....	1.31	1.03	1.12	1.29

*I. Loss on ignition minus carbonate carbon.

II. Organic carbon $\times 1.724$.

III. Organic nitrogen $\times 20$ (Kjeldahl nitrogen minus ammoniacal nitrogen).

IV. Kjeldahl nitrogen (organic nitrogen plus ammoniacal nitrogen) $\times 20$.

Analysis of organic matter

Half of the samples used in this study were analyzed by the system of proximate analysis developed by Waksman and his students and co-workers (10, 11, 12) and widely used by them and others to study organic matter changes in soils, manure, and composts. The procedures we used did not include ether and alcohol extractions; therefore, these fractions are not shown, and the water-soluble fraction is probably low. Lignin was determined by difference on the final residue as recommended for peat and composts (11) rather than for soils (12). The figures for lignin are, therefore, probably not

as reliable as they might be. For our purposes, however, this method gives very suggestive results.

The results of these analyses are shown in table 5 both on a moisture-free and on an ash-free basis. They indicate that the organic matter present in these Shantung farm manures is very similar in its chemical nature to that of soil humus, as the C/N ratio has already shown. The ash content of the 16 manures ranged from 77.10 to 92.85 per cent, with a mean of 87.01. The

TABLE 5
Proximate analysis of organic matter in Shantung farm manures
Data in per cent

SAMPLE NUMBER	MOISTURE-FREE BASIS								ASH-FREE BASIS					
	Ash	Water-soluble nitrogen	Protein	Water-soluble organic matter	Hemicellulose	Cellulose	Lignin	Total non-ash material	Water-soluble nitrogen	Protein	Water-soluble organic matter	Hemicellulose	Cellulose	Lignin
206	86.64	0.02	1.88	0.35	0.85	1.54	4.32	13.36	0.15	14.07	2.62	6.36	11.53	32.34
209	92.85	0.01	1.00	0.41	0.22	0.53	2.81	7.15	0.14	13.99	5.73	3.08	7.41	39.30
207	88.83	0.003	1.98	0.06	0.08	0.88	3.36	11.17	0.03	17.73	0.54	0.72	7.88	30.08
215	91.28	0.02	1.50	0.34	0.22	0.98	2.80	8.72	0.23	17.20	3.90	2.52	11.24	32.11
216	90.78	0.01	1.69	0.51	0.58	0.95	3.41	9.22	0.11	18.33	5.53	6.29	10.30	36.98
218	89.84	0.01	1.94	0.29	0.56	0.67	3.41	10.16	0.10	19.09	2.85	5.51	6.59	33.56
219	88.12	0.01	2.19	0.30	0.79	0.96	3.25	11.88	0.08	18.43	2.53	6.65	8.08	27.36
222	87.77	0.00	1.75	0.19	0.18	0.85	3.03	12.23	0.00	14.31	1.55	1.47	6.95	24.78
732	83.35	0.022	2.49	0.37	0.48	2.17	4.95	16.65	0.13	14.95	2.22	2.88	13.03	29.73
734	87.09	0.038	2.33	0.26	0.022	0.765	5.35	12.91	0.29	18.05	2.01	0.17	5.93	41.44
736	83.46	0.047	3.14	0.42	0.13	1.92	5.57	16.54	0.28	18.98	2.54	0.79	11.61	33.68
738	77.10	0.087	7.89	0.95	0.76	2.94	7.50	22.90	0.38	34.45	4.15	3.32	12.84	32.75
746	88.54	0.056	2.71	0.29	0.031	0.062	5.33	11.46	0.49	23.65	2.53	0.27	0.54	46.51
748	91.00	0.043	2.11	0.24	0.043	0.20	4.13	9.00	0.48	23.44	2.67	0.48	2.22	45.89
750	85.32	0.059	2.01	0.37	0.00	0.032	6.68	14.68	0.40	13.69	2.52	0.00	0.22	45.50
752	80.17	0.045	3.09	0.80	0.20	1.81	7.14	19.83	0.23	15.58	4.03	1.01	9.13	36.01
Mean...	87.01	0.03	2.48	0.38	0.32	1.08	4.56	12.99	0.22	18.50	2.99	2.59	7.84	35.50
Max....	92.85	0.087	7.89	0.95	0.85	2.94	7.50	22.90	0.49	34.45	5.73	6.36	13.03	46.51
Min.....	77.10	0.000	1.00	0.06	0.00	0.03	2.80	7.15	0.00	13.69	0.54	0.00	0.22	24.73

ash-free figures reveal the nature of the organic matter present in these manures. The first point indicated is the surprising degree of uniformity that exists between the different samples, in spite of the fact that the widest extremes of difference of raw materials going to make composts on these 32 farms were included in the analyses (table 1). The second outstanding point is that by far the greatest percentage of the organic matter present was reported as protein and lignin. From 13.69 to 34.45, average 18.50, per cent of the ash-free organic matter was protein, and from 24.78 to 46.51, mean 35.50, per cent

was lignin. The water-soluble organic matter averaged 2.99 per cent. The hemicelluloses and celluloses accounted for about 10 per cent of the ash-free organic matter, averaging 2.59 and 7.84 per cent respectively. Since animal manure, refuse, and other materials were being added to the pits right up to the time of sampling, it seems probable that the cellulose found was largely contributed by material which had not yet had time to decompose.

pH of manure

The pH values of the Tsinan series of samples were determined on fresh wet material, by means of a Beckman glass electrode pH meter, after dilution with CO₂-free water. They were found to range from 7.21 to 9.07 and averaged 8.17. Only one or two samples, in which the pH exceeded 9, had pH values which did not fall within the normal range for soils in this area. The few high pH values found probably resulted from potash added as wood ashes.

Minerals in manure

The total phosphorus content of the 32 manures ranged from 0.16 to 1.21, average 0.47 σ 0.041, per cent (table 2). When annual production per farm was calculated, the extreme range for the entire series was from 22 to 310 chin with a mean of 80.4 σ 10.1.

Total potassium present was determined by the trisodium cobaltinitrite method (13). The concentration of potassium in the different manures studied was more uniform than was the phosphorus concentration. The extreme range for the entire series was 0.67 to 1.45, mean 0.87 σ 0.027, per cent (table 2). The total yearly production varied from 29 to 1036 chin per farm with a mean of 192.8 σ 33.4 chin.

Much of both the phosphorus and potassium present in these manures undoubtedly was present in the field soil brought in as diluent.

Application of fertilizer to land

Reference to table 1 will show that the fertilizers sampled were applied to from 0.7 to 12.0 mu (0.12 to 1.98 acres), and the average area fertilized was 5.18 mu or 0.85 acres. The crops fertilized were wheat, millet, and garden plots. The actual applications calculated to chin per mu and pounds per acre are shown in table 6. The rates of application for wet materials varied between 860 and 7,590 chin per mu or 3 and 25 tons per acre with an average of 3,595 chin per mu or about 12 tons per acre. These amounts of wet material carried from 25.8 to 144, mean 65.4 σ 4.6, pounds of ammoniacal and organic nitrogen per acre and from none to 16, mean 2.9 σ 0.75, pounds of nitrate nitrogen, or a grand total of from 25.8 to 144.5, mean 68.3 σ 4.73, pounds of organic and inorganic nitrogen per acre. In terms of chin per mu, the application of nitrogen for the 32 farms ranged from 3.85 to 21.6, mean 10.21 σ 0.71; phosphorus from 3.97 to 24.6, mean 11.01 σ 0.96; and potassium from 4.72 to 52.48, mean 22.69 σ 1.81.

Accurate evaluation of the adequacy of applications of these magnitudes is prevented by a number of unknown factors. Chief of these is the degree and rapidity of availability of the major constituents present in the soil compost. It seems likely that the nitrogen is slow in becoming available, though this is somewhat offset by the fact that the applications of this fertilizer are made every year or, in some cases when soybeans are to be grown in the rotation, once every two years, and consequently the residual effect of previous applications tends to remain in the soil. This, in turn, is reduced by leaching of nitrates, though the nature and the amount of the rainfall in this area probably prevent excessive losses from this source.

The degree of availability of the phosphorus and potassium present is unknown, though it is likely also to be very low. Analyses of 11 samples of soil

TABLE 6
Rates of applications to land of fertilizer constituents on 32 Shantung farms

	CHIN PER MU				POUNDS PER ACRE			
	Maximum	Minimum	Mean	Standard deviation of mean	Maximum	Minimum	Mean	Standard deviation of mean
Total wet weight	7,590	860	3,595	30	50,800	5,700	24,731	1,742
Total organic nitrogen	21.5	3.9	9.78	0.70	144.0	25.8	65.42	4.61
Nitrate nitrogen	2.39	0.0	0.434	0.11	16.02	0.0	2.91	0.75
Total organic and inorganic nitrogen	21.6	3.85	10.21	0.71	144.54	25.79	68.33	4.73
Phosphorus, P_2O_5	24.6	3.97	11.01	0.96	164.5	26.56	73.45	6.41
Potassium, K_2O	52.48	4.72	22.69	1.81	351.15	31.58	151.97	12.16

from this region such as that used by the farmers in their compost pits averaged 0.114 per cent P_2O_5 on the oven-dry basis. Thorp (8) found 0.09 per cent of P_2O_5 in the "spoil" layer of a Shantung brown soil near Weihaiwei in Eastern Shantung. Our series of 32 farms manures, however, ranged from 0.16 to 1.21, mean 0.47, per cent phosphorus, thus showing a considerable increase in this element in the composting. Since only one sixth of the P_2O_5 in Western farm manure is considered to be easily available, it seems likely that no more than one tenth to one twentieth of the P_2O_5 in these composts is easily available. Much of the K_2O present in these soil composts also was added with the soil. Thorp found 0.45 per cent of total K_2O in the aforementioned Weihaiwei soil, and Wagner (9) found an average of 0.27 per cent potassium in 15 Shantung soils which he analyzed. Analyses of samples of soil in this laboratory averaged 0.25 per cent K_2O . Our manures, however, averaged

0.87 per cent, with a range of from 0.67 to 1.45. This increase of K_2O in the composts is due to the large amounts of straw ashes which are added to the pits. It seems probable that no more than one fourth to one third of the K_2O is easily available. The degree of availability of phosphorus and potassium in these farm manures is now being studied.

Another unknown factor is the fertilizer need of the soils to which these manures were applied. The Central Agricultural Extension Committee of the Ministry of Industry makes the following general recommendations for fertilizer applications on average soils in China (5): 15 chin of nitrogen, 12 chin of phosphorus, and 12 chin of potassium for each mu of millet; 14 chin of nitrogen, 10 chin of phosphorus, and 8 chin of potassium for each mu of wheat; and 12 to 20 chin of nitrogen, 6 to 14 chin of phosphorus, 8 to 16 chin of potassium for each mu of vegetables, depending on the crop.

If we take these recommendations as a standard for comparison and ignore the problem of availability, we find that 14, or 44 per cent, of the farms studied applied nitrogen very short of the recommendation (from one-third to a little over one-half the recommended amount); 11, or 34 per cent, made applications a little short of the recommendation (from two-thirds to three-fourths of the recommended amount); only 7, or 22 per cent, applied amounts equal to the recommendation; and none exceeded the recommendations for the crops being grown.

The picture for phosphorus is somewhat better, 14, or 44 per cent, making very short applications; 10, or 30 per cent, making slightly short applications; 4, or 13 per cent, making adequate applications; and 4, or 13 per cent, making applications in excess of the recommendation. When low availability is taken into account, however, probably none of the farms were supplying adequate amounts of P_2O_5 in their fertilizers.

The picture in relation to potassium is still better. Only 2, or 6 per cent, of the farms made applications very short of that recommended for this element; only 1, or 3 per cent, made slightly short applications; 3, or 9 per cent, made applications equal to the recommendation; and 26, or 81 per cent, applied quantities well in excess of the amount recommended. Again the limited availability of the K_2O probably results in the applications of this element being no more than enough even where the most satisfactory level is attained.

The average application of organic matter, no less than 434 chin per mu or nearly 2900 pounds per acre, for these 32 farms is surprisingly high. According to Bear (2, p. 315), this amount is almost half again as much organic matter as the average American farmer succeeds in applying in farmyard manure to his land each year. When it is remembered that virtually no crop residues are returned to Shantung soils because of the necessity of using even roots for fuel, it is obvious that these applications are far from adequate.

The indications given by these 32 farms would seem to show that the amounts of nitrogen being applied are definitely inadequate in a large percentage of the farms of Shantung. Phosphorus is being applied in inadequate

amounts, though the reserve in the semiarid North China soils is likely to be sufficient to offset this lack. The applications of K_2O very likely are sufficient to prevent this factor from being seriously limiting. The urgent need for more organic matter for North China soils is stressed by all workers [cf. Buck (4, p. 4), Thorp (8, p. 437)].

From the point of view of agricultural sanitation, this study serves to indicate that more adequate conservation of the nitrogen present in human and household wastes and an increase in the amount of organic matter which may be made available for the soil constitute the pressing fertilizer needs of this area. This is true in spite of the fact that present methods aim to conserve human excrement for use as fertilizer. There is evidence, which will be presented in succeeding papers, to show that at least half of the nitrogen consumed in food on the farm is lost. At the same time these methods make possible very high morbidity and mortality rates from fecal-borne diseases. Work now in progress holds hope that nitrogen conservation may be improved and organic matter increased as well as fecal-borne diseases reduced by the application of improved methods of composting.

SUMMARY

This paper reports studies made on "soil compost" from 32 farms in West Shantung, China. The results were as follows:

An average of about 15 metric tons of soil compost is produced annually per farm.

The concentration of total organic and inorganic nitrogen ranged from 0.196 to 1.355, with an average of 0.45 ± 0.042 , per cent. The production of nitrogen per farm ranged from 20.5 to 453.0, average 83.1 ± 13.73 , chin (500 gm.).

The total carbon averaged 5.69 ± 0.42 per cent, carbonate carbon, 0.64 ± 0.08 per cent; and organic matter based on organic carbon for the Tsinan series ranged from 4.45 to 19.34, mean 10.72 ± 1.03 , per cent.

The C/N ratios for the Tsinan series of manures ranged from 6.97 to 16.23, mean 10.41.

Organic matter present resembled soil humus, as shown by proximate analysis.

The pH of the manures ranged from 7.21 to 9.07 and fell within the range of soils of this area for the most part.

The concentration of total phosphorus as P_2O_5 ranged from 0.16 to 1.21, mean 0.47 ± 0.041 , per cent. Production per farm averaged 80.4 ± 10.11 chin, with a range from 22 to 310.

Potassium made up from 0.67 to 1.45, mean 0.87 ± 0.03 , per cent of the samples. Production per farm ranged from 29 to 1036, average 192 ± 33.4 , chin.

Though the applications of these manures varied between 3 and 25 tons per acre, averaging 12 tons, yet the low concentration and probable low availability result in inadequate fertilization in the great majority of cases.

The chief fertilizer needs of North China farms are better conservation of nitrogen and more organic matter.

REFERENCES

- (1) Association of Official Agricultural Chemists 1935 Official and Tentative Methods of Analysis, ed. 4. Washington, D. C.
- (2) BEAR, F. E. 1938 Theory and Practice in the Use of Fertilizers. John Wiley and Sons, New York.
- (3) BUCK, J. L. 1930 Chinese Farm Economy. Shanghai.

- (4) BUCK, J. L. 1937 Land Utilization in China. University of Chicago Press, Chicago.
- (5) Chinese Government Ministry of Industry 1933 Agricultural Handbook. Central Agricultural Extension Committee, Nanking. [In Chinese.]
- (6) JENKINS, S. H. 1935 Organic Manures. Imp. Bur. Soil Sci. Tech. Commun. 33.
- (7) KING, F. H. 1911 Farmers of Forty Centuries. Madison, Wis.
- (8) THORP, J. 1936 Geography of the Soils of China. National Geological Survey of China, Nanking.
- (9) WAGNER, W. 1926 Die Chinesische Landwirtschaft. Berlin.
- (10) WAKSMAN, S. A. 1936 Humus. Williams & Wilkins, Baltimore.
- (11) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: I, II. *Soil Sci.* 26: 113-137, 239-252.
- (12) WAKSMAN, S. A., AND STEVENS, K. R. 1930 A critical study of the methods for determining the nature and abundance of soil organic matter. *Soil Sci.* 30: 77-116.
- (13) WILCOX, L. U. 1937 Determination of potassium by means of an aqueous solution of trisodium cobaltinitrite in the presence of nitric acid. *Indus. Engin. Chem. Analyt. Ed.* 9: 136-138.
- (14) WINFIELD, G. F. 1937 Studies on the control of fecal-borne diseases in North China: II. The distribution of *Ascaris lumbricoides* infestations in a rural population. *Chinese Med. Jour.* 51: 502-518.

THE ROLE OF POTASSIUM IN PLANTS: III. NITROGEN AND CARBOHYDRATE METABOLISM IN POTASSIUM-DEFICIENT PLANTS SUPPLIED WITH EITHER NITRATE OR AMMONIUM NITROGEN¹

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Previous results (19, 20) in the study of the role of potassium in plants indicated that some phase of the nitrogen metabolism was adversely affected by potassium deficiency but did not definitely prove which stage was checked.

A more detailed study of the nitrogen and carbohydrate metabolism of potassium-deficient plants was therefore conducted. Harvests were made at weekly or biweekly intervals, particularly during the early stages of potassium deficiency, so that the effects of proteolysis which occur in plants exhibiting severe deficiency symptoms might be avoided. In early deficiency stages the minus-potassium plants were only slightly smaller than the complete plants; therefore, concentration effects which could produce conflicting results were partly avoided.

A series of experiments was conducted with ammonium and nitrate nitrogen to compare the effects of potassium deficiency with different sources of nitrogen. Furthermore, it was thought that the use of ammonium as a source of nitrogen would give definite information on the role of potassium in the nitrogen metabolism of the plant, since the steps of nitrate to nitrite to ammonia would be eliminated. It has been claimed that potassium deficiency checks the reduction of nitrates to ammonia (8).

Young Rutgers tomato seedlings selected for uniformity were set in sand, two plants in each crock, March 23, 1938. The plants were supplied with distilled water for one week, and were then supplied with nutrient solutions by means of a constant drip arrangement as previously described (20). The plants were divided into four series of 100 plants each, as follows: 1—plus potassium with nitrate nitrogen; 2—minus potassium with nitrate nitrogen; 3—plus potassium with ammonium nitrogen; and 4—minus potassium with ammonium nitrogen.

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In order to avoid the possible complicating effects of the drain of nutrients to the fruits, the plants were not allowed to set fruit. The ammonium plants were grown at pH 6.0, as recommended by Tiedjens (14). This pH was secured by the use of appropriate mixtures of $\text{CaH}_4(\text{PO}_4)_2$ and K_2HPO_4 and of $\text{CaH}_4(\text{PO}_4)_2$ and Na_2HPO_4 . In addition, the pots containing the plants were flushed once a day with a solution containing the respective phosphate buffers in proportions which would give a pH of 6.2, because over a period of 24 hours the initial pH tended to drop to 5.8.

The nutrient solutions (tables 1 and 2) were made up from 0.5 *M* stock solutions, with the exception of Na_2HPO_4 which was 0.25 *M* and $\text{CaH}_4(\text{PO}_4)_2$ which was 0.1 *M*.

TABLE 1
Nitrate series—partial volume molecular concentrations of nutrient solutions

SERIES	KH_2PO_4	NaH_2PO_4	$\text{Ca}(\text{NO}_3)_2$	CaCl_2	$\text{Mg}(\text{NO}_3)_2$	MgSO_4
1	.00450045	.0045	.0023	.0023
20045	.0045	.0045	.0023	.0023

TABLE 2
Ammonium series—partial volume molecular concentrations of nutrient solutions

SERIES	K_2HPO_4	Na_2HPO_4	CaCl_2	$\text{CaH}_4(\text{PO}_4)_2$	MgSO_4	$(\text{NH}_4)_2\text{SO}_4$
3	.00280023	.0023	.0023	.0014
40025	.0023	.0023	.0023	.0014
3*	.00530023	.0023	.0023	.0014
4*0044	.0023	.0023	.0023	.0014

* Daily flush.

Twenty plants from each series were taken at each harvest, at which time the height and the green weight of the plants were determined. They were then carefully fractionated into leaf and stem samples from upper, middle, and lower portions of the stem. The upper leaves in all series consisted of the uppermost cluster, which was young and contained much meristematic tissue. The middle leaves in all series were taken from the compound leaf nearest the middle of the plant. Usually the three blades at the extreme end of the leaf were sufficient for analysis. The lower leaves, selected from the complete plants, were comparable in age to those plants of the potassium-deficient ammonium or nitrate series. The lower leaf, at the base of the potassium-deficient plant, which had not died was selected. The terminal blades were then used for analysis, as in the case of the middle leaves. Three centimeters of the extreme tip, middle, and base of the stems were used for stem aliquots. By this method of fractionation it was hoped to separate meristematic from mature and very old tissue, and thus accurately study the effects of potassium in the various regions of the plant.

CHEMICAL METHODS

On account of the number of harvests and the large number of plant fractionations, it was necessary to preserve the harvested tissue. After the plants were fractionated at the various harvests, therefore, each plant fraction was immediately weighed and rapidly dried in an oven at 75–80° C. Though this method may be open to objection from the standpoint of the possible effects on the nitrogen fractions, Vickery et al. (17) and Clark (2) have used it in extensive investigations and give data to prove the validity of the drying method for tobacco and tomato tissue. The dried plant material was weighed and the percentage dry weight calculated from the original fresh weight. Because of the small sample the tissue was ground with a mortar and pestle. The tomato leaves could be ground to a very fine powder. The stem tissue was not so finely ground, but the results of replicate nitrogen and carbohydrate analyses showed that no loss in accuracy was entailed.

On account of the very small amounts of dried tissue available in most cases, semimicroanalytical methods were used to determine nitrogen and carbohydrate fractions.

Nitrogen fractions

Total nitrogen. Total nitrogen was determined by a micromodification of the Ranker (10) method. When nitrates were present, 10–20 mgm. of dried tissue was placed in a microdigestion flask, 1 cc. of sulfuric-salicylic acid mixture (30 gm. of salicylic acid per liter of concentrated sulfuric acid) and 3 cc. of sulfuric acid-selenium mixture (7 cc. of selenium oxychloride per liter of concentrated sulfuric acid) were added. A few crystals of potassium sulfate and about 0.1 gm. of sodium thiosulfate were then added. The flasks were heated with a very small flame of a microburner in order to avoid excessive frothing. The flame was gradually increased and finally turned on full. A complete digestion usually took from one to two hours. The procedure was similar for tissue containing no nitrates, but no sulfuric-salicylic acid mixture or sodium thiosulfate was added, a full flame could be used at once, and digestions were usually completed within half an hour. The contents of the flasks were washed into a Pregl microdistillation apparatus, and 10–12 cc. of approximately 50 per cent NaOH was added. The ammonia was distilled into 0.02 *N* HCl and titrated with 0.02 *N* NaOH by means of a microburette. Blank determinations were run each day. Duplicates usually checked within 0.1–0.2 per cent on an absolute basis. The method was checked on samples, the total nitrogen of which had been determined by macro-Kjeldahls. Very satisfactory agreement was obtained between the micro- and the macroresults.

Nitrate nitrogen. Approximately 50-mgm. duplicate samples each were placed in a 100-cc. beaker to which 20 cc. of water was added. The suspension was stirred on a boiling water bath for 15 minutes, and 0.1–0.2 gm. of charcoal was added to remove coloring matter. After a few minutes the sus-

pension was filtered into an evaporating dish. The residue was washed several times with a few milliliters of hot water until the washings gave no test for the nitrate ion with diphenylamine. The filtrate in the dish was evaporated to dryness on a steam bath, and nitrate determined by the phenol-disulfonic acid method (6). Nitrates were not found in the plants grown with ammonium nitrogen, and hence were not determined on such samples.

Soluble organic nitrogen. In a 150-cc. beaker, 0.5–1 gm. of dried material was stirred to a paste with a small quantity of water. Approximately 50 cc. of water was added, and the suspension was stirred constantly at 80°C. for 10 minutes. According to Vickery et al. (18), this procedure will completely extract soluble organic fractions from plant material and will not hydrolyze the amide glutamine. The suspension was filtered with suction on a Büchner funnel, and the residue was washed several times with a few milliliters of hot water until the washings gave a negative test for nitrates with diphenylamine or, in the case of plants with ammonium nitrogen, a negative Nessler's test for ammonia. The filtrate was made up to 100 cc., a few drops of toluene were added, and the solutions were kept in the cold storage room until analyzed, within two to three days.

Total soluble nitrogen. Five-milliliter aliquots were placed in a microdigestion flask and carefully reduced in volume to 1 or 2 cc. over a free flame. The flasks were then put in a boiling water bath and connected to a water suction pump. The pump was cautiously turned on, and gradually the vacuum was increased. The material in the flasks could be evaporated to dryness in less than five minutes by this method. From this point the procedure was the same as for total nitrogen.

Ammonium nitrogen. Ten-milliliter aliquots were used for the determination of ammonium nitrogen by the method of Pucher et al. (9). The ammonia was caught in 0.01 *N* HCl and titrated with 0.01 *N* NaOH by means of a microburette.

Total amide nitrogen. Depending upon the amount of free ammonia found previously, 10- or 20-cc. aliquots were hydrolyzed for 3 hours on a boiling water bath with 6 *N* H₂SO₄ according to the method of Pucher et al. (9). The acid was neutralized, and the free ammonia was determined as above. Total amide nitrogen was calculated as the difference between the total ammonia found after hydrolysis and the ammonium nitrogen previously determined.

Amino nitrogen. The alkaline residue remaining from the amide determination was made slightly acid with glacial acetic acid and washed into a 150-cc. beaker. The solution was evaporated below 25 cc. and then transferred to a 25-cc. volumetric flask and made to volume. Ten-milliliter duplicates were used to determine amino nitrogen by the Van Slyke method.

Other fractions. Protein nitrogen was calculated as the difference between total nitrogen and total soluble nitrogen. Soluble organic nitrogen was calculated as the difference between total soluble nitrogen and nitrate nitrogen.

Carbohydrate fractions

Duplicate 0.1-gm. samples were extracted with 80 per cent alcohol as previously described (20). The alcoholic suspension was filtered and washed with hot 80 per cent alcohol. Water was added to the alcoholic filtrate and the alcohol boiled off. The filtrate was made up to a volume of 50 cc., cleared with lead acetate, and delead with sodium oxalate as in the previous experiment. Reducing sugars and total sugars were determined on 5-cc. aliquots by the method of Van der Plank (16). Sucrose was calculated as the difference between total sugars and reducing sugars.

The residue from the alcoholic extract was treated for starch as described previously (20). Since hemicellulose had been found to be low in tomatoes, the hemicellulose fractions were not separated from the starch fractions. The final solution was made up to a volume of 100 cc., cleared, and delead, and 5-cc. aliquots were taken for glucose determinations according to the method of Van der Plank (16). Starch plus hemicellulose was calculated in the terms of the glucose found.

RESULTS

Growth results

The growth of the plants of series 1 (nitrate and potassium) and series 2 (nitrate without potassium) was similar to that of the plants in previous experiments (19, 20). The plants in both series rapidly turned green after receiving the nutrient solutions, and for some time both series made almost equal growth. By April 19, however, 20 days after receiving the initial minus-potassium solution, the plants in series 2 showed retarded growth, hardness, and yellowing of the leaves. By May 5, 36 days after receiving the minus-potassium nutrient solution, the plants of series 2 were showing typical rust-colored spots on the lower leaves. At the last harvest, May 27, many of the lower leaves of these plants were dying, exhibiting the extreme symptoms of potassium deficiency.

The plants of series 3 (ammonium and potassium) and series 4 (ammonium without potassium) displayed a type of growth which differed markedly from the nitrate series. These plants turned green very rapidly when given the nutrient solutions, but made a softer, less vigorous growth than the plants of the nitrate series. The plants of both series 3 and 4 made a similar growth until April 16. Then all the leaves on the minus-potassium plants very suddenly began to die. Rust-colored spots were not noticed on these leaves, nor was injury restricted to the lower leaves. By April 19, very marked symptoms had developed. After these plants were harvested, a new series of plants supplied with ammonium nitrogen was started. The tomato seedlings were set in sand April 21 and given nutrient solution April 28. By May 5, the new plants of series 4 showed the early breakdown symptoms of the previously

grown plants. By May 27, at the last harvest, more than two-thirds of the leaves on these plants were dead. At this time the plants of series 3 were in excellent condition, the foliage being dark green and the stems succulent. The potassium plants were much larger than the potassium-deficient plants supplied with ammonium. The heights and green weights of both the nitrate and ammonium plants over the course of the experiment are shown in table 3.

The results of the April 19 harvest show clearly that the nitrate series made better growth than the ammonium series. Indeed, the nitrate plants without potassium were larger at this time than the ammonium plants with potassium, although it is doubtful whether this relationship would exist over a prolonged period. In both the nitrate and ammonium series the effect of potassium deficiency was to lower greatly the green weights and to a less degree the heights of the plants. The very rapid effect of potassium deficiency on the plants supplied with ammonium may be illustrated by the following

TABLE 3
Average height and green weight of plants grown with ammonium and nitrate nitrogen with and without potassium

DATE	SERIES 1		SERIES 2		SERIES 3		SERIES 4	
	Height	Weight	Height	Weight	Height	Weight	Height	Weight
	cm.	gm.	cm.	gm.	cm.	gm.	cm.	gm.
3/30/38	26.0*	28.4*
4/19/38	52.0	106.5	44.3	72.8	40.3	55.5	38.3	49.6
4/28/38	62.0	185.8	53.5	116.4	23.3*	19.8*
5/ 5/38	76.5	283.7	69.8	171.7	23.5	24.3	22.8	22.9
5/13/38	89.3	434.3	81.0	211.8	38.8	53.3	33.5	29.8
5/27/38	103.8	547.7	95.2	294.3	53.3	85.2	43.5	36.6

* Original harvest just before application of initial nutrient solution.

facts: The plants of series 2 had slightly more than half the green weight of those of series 1 after 58 days. At 29 days the corresponding plants of series 4 had less than half the green weight of those of series 3.

From these results, it would seem that the rapid accumulation of ammonium in the potassium-deficient ammonium plants is responsible for the rapid deterioration of the tissue. Moreover, these effects were not due to an unfavorable pH of the nutrient solution. The effects of ammonium in potassium deficiency are clearly depicted in plate 1.

It has been shown that tomato plants supplied with ammonium nitrogen will not grow well because they cannot assimilate ammonium nitrogen at a pH below 6.0 (14). Both the complete and the minus-potassium plants at pH 5.0 were hard, yellow, and stunted. There was, however, little leaf breakdown. Indeed, as can be seen from plate 1, there were only slight differences in size and appearance between the complete and the minus-potassium ammonium plants grown at pH 5.0. Very different was the situation in the case

of complete and potassium-deficient plants grown at pH 6.0. The ammonium plants receiving complete potassium were growing vigorously. The tomato plants which had received no potassium not only made much slower growth, but in addition almost two-thirds of the leaves were dead or dying. The breakdown symptoms on the leaves of the minus-potassium plants appeared very suddenly and spread rapidly. The symptoms were different from those appearing on potassium-deficient plants supplied with nitrate and appeared in a much shorter period. It is evident, then, that these toxic effects appeared only in potassium-deficient plants grown at a pH at which ammonium ions could be assimilated. The toxic effects could not be due to the external ammonium concentrations, since the plants with potassium at pH 6.0 grew well, or to potassium deficiency alone, since potassium-deficient plants supplied with nitrate had different deficiency symptoms which did not appear in so short a time. The high internal concentration of ammonium ions of the potassium-deficient plants was undoubtedly responsible for the severity of the deficiency symptoms. The fact that ammonium ions absorbed by the potassium-deficient tomato plants were not readily assimilated soon resulted in an internal concentration of ammonium, the nature of which is discussed elsewhere in this paper.

Elongation of the stem was much less affected than green weight by potassium deficiency in both nitrate and ammonium experiments, a finding which agreed with results of the previous experiment. This diminution in green weight is due partly to the failure of potassium-deficient plants to synthesize food reserves.

Nitrogen metabolism

Some of the results for the nitrogenous fractions³ are shown in tables 4 and 5, and in figure 1. The distribution of nitrogen in the plant fractions varied widely. Protein nitrogen was much higher in the leaves than in the stems. The upper leaves had a higher protein content than the middle leaves, which in turn had a higher protein content than the lower leaves. The same relationship held for the upper, middle, and lower stem tissue. The stems were much higher in soluble organic nitrogen than the leaves. The middle of the stems had the highest soluble organic nitrogen concentration. The tips of the stems were, as a rule, higher in soluble organic nitrogen content than the base of the stems. The differences among the three leaf fractions were not so marked as in the stem fractions.

The middle stem portions seem to contain the greatest concentrations of reserves or temporarily unassimilated nutrients. Ammonia, amide, amino, and nitrate nitrogen are very high in this plant fraction, and soluble sugars and starch are also present in high concentrations.

Nitrate was found to be very high in all the stem fractions of plants grown

³ More detailed data supplementing those presented in this discussion may be found in the author's thesis filed in the library of Rutgers University.

TABLE 4

Comparison of the chemical composition of tomato plants receiving adequate and potassium-deficient nutrient solutions containing nitrogen only in nitrate form

Data expressed as percentage of dry matter

	20 DAYS				36 DAYS				58 DAYS			
	Sol. org. N Protein N	NH ₃ -N	Total sugars	Total carbo- hydrates	Sol. org. N Protein N	NH ₃ -N	Total sugars	Total carbo- hydrates	Sol. org. N Protein N	NH ₃ -N	Total sugars	Total carbo- hydrates
Upper leaves + K...	.20	.033	3.14	10.88	0.24	3.85	16.25	0.21	.034	4.55	20.33
Upper leaves - K...	.20	.037	4.41	15.03	0.25	.021	6.51	17.77	0.18	.011	4.01	20.65
Middle leaves + K...	.11	.051	2.77	11.37	0.27	.029	3.00	10.62	0.38	.032	3.16	10.98
Middle leaves - K...	.25	.032	3.44	12.44	0.37	.059	4.21	9.79	0.42	.050	4.73	11.85
Lower leaves + K...	.11	.048	1.43	5.23	0.32	.035	1.67	6.45	0.41	.025	1.29	7.07
Lower leaves - K...	.36	.038	1.94	7.94	0.46	.049	3.95	8.93	0.31	.043	4.55	10.55
Upper stems + K...	.15	.029	2.81	10.11	0.32	.045	7.96	14.88	0.42	.027	7.47	16.49
Upper stems - K...055	4.39	11.81	0.45	.083	8.85	17.47	0.35	.050	6.59	13.89
Middle stems + K...	.51	.030	4.77	12.67	0.58	.061	8.85	16.47	1.36	.110	11.13	24.11
Middle stems - K...	.72	.061	6.90	13.83210	9.09	18.83	1.93	.390	5.27	11.87
Lower stems + K...	.62	.018	10.10	28.46	0.66	.049	9.50	26.40	1.08	.130	7.89	28.13
Lower stems - K...	.60	11.77	35.03	1.03	.140	8.29	29.25	1.64	.300	6.59	22.45

TABLE 5

Comparison of the chemical composition of tomato plants receiving adequate and potassium-deficient nutrient solutions containing nitrogen only in ammonium form

Data expressed as percentage of dry weight

	7 DAYS				15 DAYS				29 DAYS			
	Sol. org. N Protein N	NH ₃ -N	Total sugars	Total carbo- hydrates	Sol. org. N Protein N	NH ₃ -N	Total sugars	Total carbo- hydrates	Sol. org. N Protein N	NH ₃ -N	Total sugars	Total carbo- hydrates
Upper leaves + K...	0.25	.064	2.49	20.19	0.33	.032	5.73	19.71	0.22	.034	1.25	12.75
Upper leaves - K...	0.21	.067	4.67	23.67	0.25	.051	2.79	18.13	0.32	.110	2.13	15.07
Middle leaves + K...	0.32	.076	3.26	30.60	0.46	.120	7.37	20.95	0.39	.120	3.81	11.35
Middle leaves - K...	0.42	.056	4.81	34.21	0.46	.300	4.71	15.45	0.43	.510	2.00	9.50
Lower leaves + K...	0.46	.085	6.77	15.87	0.44	.160	3.00	9.32
Lower leaves - K...
Upper stems + K...	0.35	.077	2.59	9.31	0.46	.015	5.11	13.89	0.45	.180	0.75	6.87
Upper stems - K...	0.82	.089	7.03	16.93	0.65	.200	2.83	12.17	1.09	.210	0.85	7.57
Middle stems + K...	0.86	.023	14.66	29.80	1.39	.110	10.42	18.44	0.91	.220	3.91	12.81
Middle stems - K...	1.13	.062	21.80	38.70	1.43	.360	8.35	18.01	1.50	.560	3.06	10.56
Lower stems + K...	0.64	.029	13.77	36.43	1.03	.070	12.22	25.96	0.76	.140	4.01	20.01
Lower stems - K...	1.04	.037	13.28	38.94	0.85	.120	11.19	24.93	1.40	.250	5.21	14.95

with nitrate nitrogen. In the leaves, however, it was very low and, indeed, was absent in most cases from the leaves of the upper portions of the stems. Since the upper leaves are probably centers of intense synthetic activity,

it is not surprising to find nitrate nitrogen absent, but the high concentrations present in the meristematic tissue of the upper stem region would seem to show that the synthetic activities in this region differ in nature or intensity from those in the tip leaves.

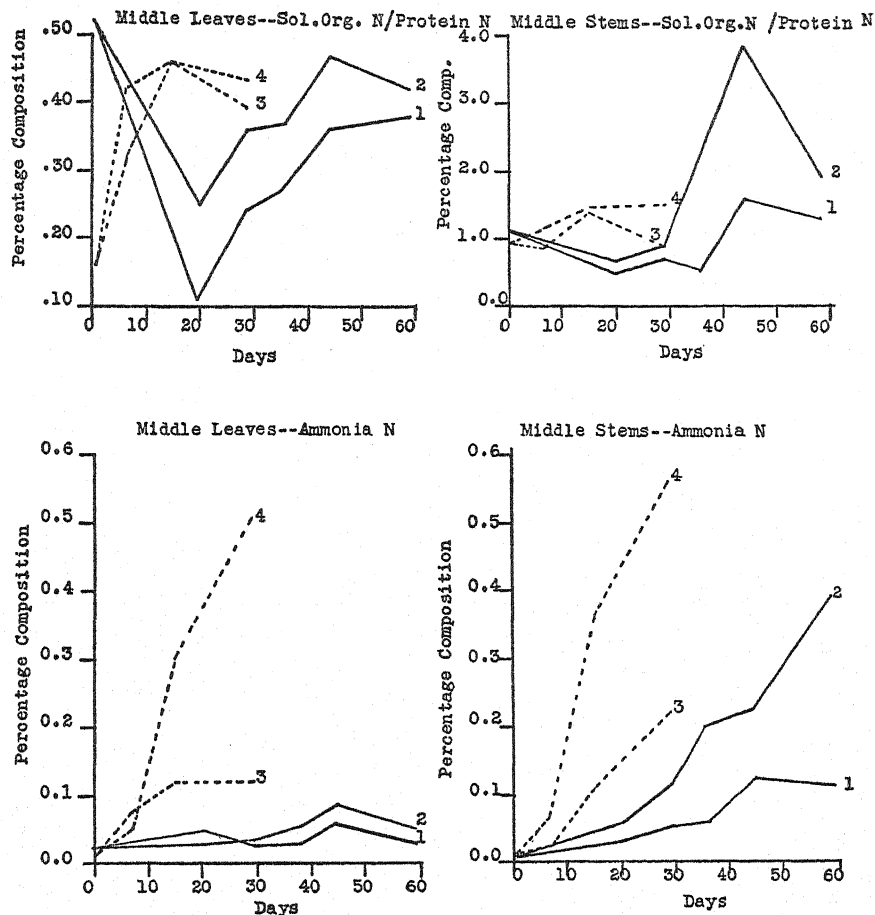


FIG. 1. NITROGENOUS FRACTIONS OF TOMATO PLANTS SUPPLIED WITH NITRATE AND AMMONIUM NITROGEN NUTRIENT SOLUTIONS WITH AND WITHOUT POTASSIUM

- 1—Nitrate nitrogen with potassium.
- 2—Nitrate nitrogen without potassium.
- 3—Ammonium nitrogen with potassium.
- 4—Ammonium nitrogen without potassium.

The distribution of nitrogen in general, as mentioned above, was not altered by the source of nitrogen (nitrate or ammonium), although the plants supplied with ammonium nitrogen tended to have higher soluble organic concentrations and lower protein concentrations than the nitrate plants. Neither did po-

tassium deficiency alter the general nitrogen distribution, but did cause very noticeable differences in the concentrations of the various nitrogenous fractions.

The minus-potassium plants in both the nitrate and ammonium series in general showed a higher total nitrogen content throughout the course of the experiments. On the other hand, the concentration of protein nitrogen in the first weeks of the experiment was usually lower in the potassium-deficient plants. During the later stages of the experiment, protein nitrogen increased. This increase in protein nitrogen may be brought about by the smaller size of the potassium-deficient plants in comparison to the plants receiving potassium. Therefore, the decreased concentration in protein nitrogen in the early period of the experiment when the potassium-deficient plants were not much smaller than the plants receiving potassium, may be very significant. Moreover, the ratio of soluble organic nitrogen to protein nitrogen was almost always much higher in the potassium-deficient plants than in the plants receiving potassium in both the nitrate and ammonium series. This ratio was almost the same, however, in the meristematic upper leaves, and indeed the value of this ratio remained remarkably constant throughout the course of the experiment.

Soluble organic nitrogen was much higher in the potassium-deficient plants of both the nitrate and ammonium series than in those supplied with the complete nutrient solutions. During the later weeks of the experiment this accumulation may have been partly due to the proteolysis occurring in the potassium-deficient leaves. This increase in soluble organic nitrogen, however, was also found in the early stages of potassium deficiency when leaf proteolysis was not occurring at a rapid rate. It was least apparent in the upper leaves of the minus-potassium plants.

No accumulation of nitrates could be found in the potassium-deficient nitrate plants. Ammonium, amide, and amino nitrogen did accumulate, particularly in the middle stems, whereas there was a decrease, in many cases, in the actual protein, and in almost all cases in the relative protein concentration. In the ammonium series, ammonium, amide, and amino nitrogen accumulated, and protein decreased. It is very significant that in both the nitrate and the ammonium series, carbohydrates accumulated. This accumulation of soluble organic nitrogenous fractions and carbohydrates must have begun in the very early stages of potassium deficiency, before any external signs of this deficiency appeared. The evidence presented clearly proves that limiting potassium retards nitrogen metabolism of the tomato plant, and indirectly proves that this occurs before the carbohydrate metabolism is affected. Carbohydrates would not accumulate if the nitrogen metabolism were not affected first. The accumulation of carbohydrates in the ammonium series and of ammonium in both the ammonium and nitrate series, and the failure of nitrates to accumulate in the minus-potassium series (actually the nitrate concentration was lower than that in the plants supplied with potassium), would seem to prove that the reduction of nitrates to ammonia is not affected

by potassium deficiency nor is it the cause of the accumulation of carbohydrates by the potassium-deficient plants. The hypothesis that potassium deficiency interferes with the synthesis of protein from an elaborated source of nitrogen is supported by the results of this investigation, i.e., accumulation of soluble organic nitrogenous and carbohydrate fractions in both the nitrate and ammonium series coupled with the decrease in actual and, particularly, relative protein concentrations in both series. This is elaborated further in the discussion.

Carbohydrate results

Results for some of the carbohydrate fractions are shown in tables 4 and 5. The distribution of these fractions varied according to the part of the plant analyzed. The stems were much higher in the reducing sugars, sucrose, and starch than the leaves. The lower stems usually had the highest total carbohydrate content, due to their large reserves of starch. The upper leaves had the highest total carbohydrate content of the leaf fractions, due also to the high starch concentrations present. There was little sucrose present in the tomato leaves, almost all of this fraction being concentrated in the middle and lower stems.

Whether nitrate or ammonium was supplied or whether a deficiency of potassium existed did not alter the general carbohydrate distribution. As the experiment progressed, carbohydrates tended to increase with nitrate and to decrease in the tomatoes grown with ammonium. This result was to be expected, since ammonium seemed to be assimilated much more rapidly than nitrate by the plant, and consequently carbohydrates were utilized more rapidly by the ammonium plants.

The potassium-deficient plants in both the nitrate and ammonium series accumulated carbohydrates in the first few weeks of the experiment. The light conditions were very good during the course of this experiment, and as a result the potassium-deficient plants continued to accumulate carbohydrates until the last weeks of the experiment, when the carbohydrate concentrations began to decrease in a manner similar to that previously found (20). In the nitrate series, carbohydrates decreased more in the minus-potassium plants than in the potassium plants after receiving nutrient solution for 36 days. The ammonium series, on the other hand, began to show a similar decrease after 15 days. The tendency was for the carbohydrate concentrations in the leaves of the potassium-deficient plants to be maintained at the expense of the stems, where the carbohydrate concentrations fell to low values by the end of the experiment. The evidence indicates that reducing-sugar content in all the potassium-deficient plant fractions was maintained in the final stages of the experiment at the expense of starch and sucrose. These latter carbohydrates were very low in the stems of the potassium-deficient plants of both series in the last weeks of the experiment, whereas reducing sugars were maintained at comparatively high concentrations.

The explanation for the accumulation of carbohydrates in potassium-deficient tomato plants is to be found in the effects of potassium deficiency on the nitrogen metabolism of these plants. It is difficult to say whether the final decrease in the carbohydrate content of such plants is due to the direct effect of potassium deficiency on CO_2 assimilation and carbohydrate synthesis or to the effect of the disrupted nitrogen cycle which is caused by potassium deficiency. This point will be discussed later in this paper. It is probable from the results of this experiment that the carbohydrate metabolism is not disturbed in potassium-deficient plants until the nitrogen metabolism is altered. For all practical purposes, both phases of the plant metabolism will be affected simultaneously.

Very little work has been done on the effects of potassium on plants grown with ammonium as the source of nitrogen. Turtshin (15) and, in a very recent article, Schropp and Arenz (13) report that ammonium has a very toxic effect on potassium-deficient plants. The results of the present studies confirm these findings that the ammonium nitrogen increases in minus-potassium tomato plants. The symptoms associated with potassium-deficient tomatoes grown with ammonium nitrogen were much more severe than when grown with nitrate nitrogen: the first external sign was a very rapid breakdown of leaf tissue, which was widespread, in contrast with its confinement to the lower leaves of potassium-deficient nitrate plants. The cause of this breakdown undoubtedly was the high internal concentration of ammonium ions which could not be assimilated by the potassium-deficient plants. This is in agreement with results published by Turtshin (15).

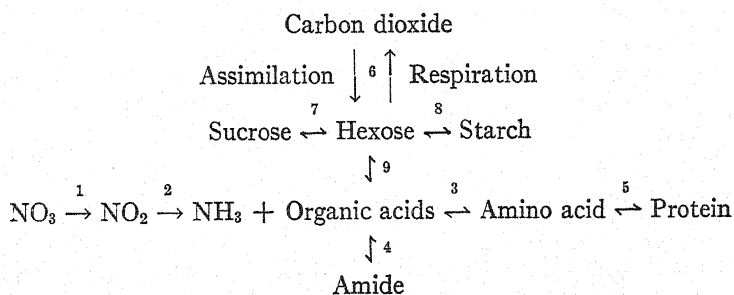
Effects of potassium on nitrogen and carbohydrate metabolism

The literature is replete with conflicting reports as to the effects of potassium on nitrogen and carbohydrate metabolism. Burrell (1), Nightingale et al. (8), Hartt (7), Schmalfuss (12), Turtshin (15), and Wall (19) have reported that potassium directly affects nitrogen metabolism. Gregory and co-workers in England (3) claim that potassium has no direct effect on nitrogen metabolism. Virtually all workers agree that soluble organic nitrogen fractions increase in potassium-deficient plants. Those who claim that potassium directly affects the nitrogen metabolism believe that this increase is due to some interference with protein synthesis. Gregory and co-workers claim that this increase in soluble organic nitrogen is due to abnormally rapid synthesis and hydrolysis of protein. One very important difference between the two schools of opinion is that Nightingale et al., Turtshin, Hartt, and Wall noticed initial accumulation of carbohydrates in potassium-deficient plants, whereas Gregory and co-workers did not find this initial accumulation in their experimental plants. The only other way in which carbohydrates could have accumulated under the experimental conditions prevailing in the potassium-deficiency studies mentioned above was through some interference in the nitrogen metabolism.

Workers in England have claimed that the accumulation of soluble organic nitrogenous fractions found by other investigators was due only to the proteolysis in potassium-deficient leaves and was not of synthetic origin. Analytical data were obtained from plants in which potassium deficiency gradually increased. In the early stages of this experiment, abnormal proteolysis could not have played a prominent role in these plants, for carbohydrate accumulation was found in both nitrate and ammonium plants grown with no potassium. Proteolysis does not occur with abnormal rapidity if abundant carbohydrates are present. With the potassium-deficient nitrate and ammonium plants an increase in soluble organic nitrogenous fractions was found to be correlated with the accumulation of carbohydrates. In the final stages the carbohydrate accumulations of both potassium-deficient series began to fall, while soluble organic nitrogenous fractions increased very rapidly. Undoubtedly proteolysis was taking place at this point. This fact, however, in no way disproves that the initial accumulation of soluble organic nitrogen in the potassium-deficient series was of synthetic origin.

Since the accumulation of carbohydrates and of soluble organic nitrogenous fractions was found simultaneously, it is very probable that interference with the nitrogen metabolism occurred before the carbohydrate metabolism was affected. The fact that, with the exception of the young leaves from the upper part of the stems, the protein concentrations of the potassium-deficient plants grown with nitrate and ammonium nitrogen were lower than the protein concentrations of the complete nutrient series seems to prove this point definitely. The leaves from the upper stems had a normal protein and soluble organic nitrogen content, probably due to the high concentration of potassium in the young meristematic tissue.

✓ The results of the experiments seem to indicate clearly that nitrogen metabolism in the tomato plant is disrupted by potassium deficiency. The following simple equation may serve to clarify the interrelations of the nitrogen and carbohydrate metabolism in the case of potassium deficiency:



It has been shown by the previously mentioned experiments that in the early stages of potassium deficiency with either nitrate or ammonium as the source of nitrogen, ammonia, amide, amino nitrogen and carbohydrates accumulated, while proteins decreased. The present knowledge of the synthesis

of protein in the green plant is far from complete. The belief is held at present that proteins are synthesized from amino acids and that amides are a form in which excess ammonia may be stored until needed. If the process represented by the equation is checked at 5, the synthetic reactions at 3 and 9 will be halted, on the assumption that the mass action law is applicable. Therefore, amino acids, ammonia, amides, hexose, sucrose, and starch will accumulate, while the protein will decrease. These results are in accord with the experimental observations. There is little evidence at present to show that reactions 1 and 2 are reversible in growing plants. Actually no nitrate accumulation was found in potassium-deficient plants. Gregory and Richards (4), Richards (11), and Gregory and Sen (5) have shown, however, that carbon dioxide assimilation decreases and respiration increases in potassium-deficient plants. This would account for the final decrease in the carbohydrate content of potassium-deficient plants which has been found by many workers. These results are also in accord with the evidence found in these experiments where the initial carbohydrate accumulations finally were reduced below the carbohydrate concentrations of plants supplied with potassium. There was some evidence to show that starch and sucrose decreased first, thus maintaining for some time the hexose concentration. Under such low carbohydrate conditions considerable proteolysis undoubtedly takes place. If plants are analyzed at this stage it is impossible to determine whether the soluble organic products found are of synthetic or hydrolytic origin.

The final effect of potassium deficiency on metabolism is to decrease the carbohydrate concentration. Since the results of these investigations seem to give strong evidence that the nitrogen metabolism is affected prior to the carbohydrate metabolism, it is difficult to say whether the final decrease in carbohydrate concentration is a direct or an indirect effect of potassium deficiency. The interference with metabolism brought about by the effects of potassium deficiency on the nitrogen metabolism may possibly account for the final reduction in carbon dioxide assimilation. The protoplasm in the cells certainly cannot function as effectively as that in plants grown with a complete solution.

The stage at which the nitrogen metabolism is affected by potassium deficiency seems to be that of the condensation of amino acids to protein. The fact that carbohydrate accumulations occurred in potassium-deficient plants grown with ammonium nitrogen, along with the fact that ammonium accumulated in potassium-deficient plants of both the nitrate and the ammonium series, while nitrates did not accumulate in potassium-deficient nitrate plants, seems at variance with, but does not disprove, the hypothesis that the reduction of nitrates to ammonia is affected by potassium deficiency, as was suggested by Nightingale et al. (8).

SUMMARY

Young Rutgers tomato seedlings were grown with nutrient solutions containing nitrate and ammonium nitrogen, with and without potassium. The

plants were harvested at weekly or biweekly intervals over a period of 2 months. The nitrate plants made better growth than the corresponding ammonium plants. Potassium-deficient plants grown with nitrate gradually showed typical deficiency symptoms similar to those discussed in a previous experiment. The potassium-deficient plants grown with ammonium suddenly developed leaf-breakdown symptoms totally dissimilar to those of the potassium-deficient nitrate plants. The leaves on these ammonium plants died very rapidly. From the chemical evidence, the cause of this rapid breakdown was the toxic effect of high internal ammonium concentrations in the potassium-deficient plants.

The results of chemical analyses demonstrated that potassium-deficient plants of both the nitrate and the ammonium series accumulated ammonia, amide, and amino nitrogen, while the protein concentration decreased. Simultaneously the plants showed an initial carbohydrate accumulation which finally decreased and fell to lower values than in plants supplied with complete potassium. The evidence seems to show that protein synthesis from an elaborated form of nitrogen is affected by potassium deficiency. This would account satisfactorily for the increased ammonia, amide, and amino nitrogen concentrations, for the decrease in protein, and for the initial accumulation of carbohydrates in potassium-deficient tomato plants.

The analytical data point strongly to the fact that the nitrogen metabolism is affected by potassium deficiency prior to the carbohydrate metabolism. The final drop in carbohydrate content of potassium-deficient plants may be due to a direct need for potassium in CO_2 assimilation or to the indirect effects on protoplasm brought about by the interference with the nitrogenous metabolism of potassium-deficient plants.

REFERENCES

- (1) BURRELL, R. C. 1926 Effect of certain deficiencies on nitrogen metabolism of plants. *Bot. Gaz.* 82: 320-329.
- (2) CLARK, H. E. 1936 Effect of ammonium and nitrate nitrogen on the composition of the tomato plants. *Plant Physiol.* 11: 5-24.
- (3) GREGORY, F. G. 1937 Mineral nutrition of plants. *Ann. Rev. Biochem.* 6: 557-578.
- (4) GREGORY, F. G., AND RICHARDS, F. J. 1929 Physiological studies in plant nutrition: I. The effect of manurial deficiency on the respiration and assimilation rate in barley. *Ann. Bot.* 43: 119-161.
- (5) GREGORY, F. G., AND SEN, P. K. 1937 Physiological studies in plant nutrition: VI. The relation of respiration rate to the carbohydrate and nitrogen metabolism of the barley leaf as determined by nitrogen and potassium deficiency. *Ann. Bot.* 1: 521-561.
- (6) HARPER, H. J. 1924 The accurate determination of nitrates in soils—phenoldisulfonic acid method. *Indus. and Engin. Chem.* 16: 180-183.
- (7) HARTT, C. E. 1934 Some effects of potassium upon the amounts of protein and amino forms of nitrogen, sugars and enzyme activity of sugar cane. *Plant Physiol.* 9: 452-490.
- (8) NIGHTINGALE, G. T., SCHERMERHORN, L. G., AND ROBBINS, W. R. 1930 Some effects of potassium deficiency on the histological structure and nitrogenous and carbohydrate constituents of plants. *N. J. Agr. Exp. Sta. Bul.* 499.

- (9) PUCHER, G. W., VICKERY, H. B., AND LEAVENWORTH, C. E. 1935 Determination of ammonia and of amide nitrogen in plant tissue. *Indus. and Engin. Chem., Analyt. Ed.* 7: 152-156.
- (10) RANKER, E. R. 1927 A modification of the salicylic-thiosulphate method suitable for the determination of total nitrogen in plant solutions and soil extracts. *Jour. Assoc. Off. Agr. Chem.* 10: 230-251.
- (11) RICHARDS, F. J. 1932 Physiological studies in plant nutrition: III. Further studies of the effect of potash deficiency on the rate of respiration in barley. *Ann. Bot.* 46: 367-388.
- (12) SCHMALFUSS, K. 1932 Untersuchungen über den Eiweissstoffwechsel von Kalimangelpflanzen. *Phytopath. Ztschr.* 5: 207-249.
- (13) SCHROPP, W., AND ARENZ, B. 1939 Über die Wirkung des Kaliums bei der Ernährung der Pflanzen mit Nitrat- und Ammoniakstickstoff. *Ernähr. Pflanze.* 35: 97-106.
- (14) TIEDJENS, V. A. 1934 Factors affecting assimilation of ammonium and nitrate nitrogen particularly in tomato and apple. *Plant Physiol.* 9: 31-57.
- (15) TURTSCHIN, T. W. 1934 Einfluss des Kalis auf den Stickstoff und Kohlenhydratenwechsel in Pflanzen. *Ztschr. Pflanzenernähr., Düngung u. Bodenk.* 35A: 343-357.
- (16) VAN DER PLANK, J. E. 1936 The estimation of sugars in the mangold. *Biochem. Jour.* 30: 457-469.
- (17) VICKERY, H. B., ET AL. 1935 Chemical investigations of the tobacco plant: V. Chemical changes that occur during growth. *Conn. Agr. Exp. Sta. Bul.* 374.
- (18) VICKERY, H. B., ET AL. 1937 Chemical investigations of the tobacco plant: VI. Chemical changes that occur in leaves during culture in light and in darkness. *Conn. Agr. Exp. Sta. Bul.* 399.
- (19) WALL, M. E. 1939 The role of potassium in plants: I. Effect of varying amounts of potassium on nitrogenous, carbohydrate, and mineral metabolism in the tomato plant. *Soil Sci.* 47: 143-161.
- (20) WALL, M. E. 1940 The role of potassium in plants: II. Effect of varying amounts of potassium on the growth status and metabolism of the tomato plant. *Soil Sci.* 49: 315-331.

PLATE 1

TOMATO PLANTS SUPPLIED WITH AMMONIUM NITROGEN, WITH AND WITHOUT POTASSIUM
AT pH 6.0 AND 5.0

1. Plus potassium—pH 6.0. 2. Plus potassium—pH 5.0. 3. Minus potassium—pH 6.0.
4. Minus potassium—pH 5.0.



1. 10 g. 100 g. 5.0 -K 1.0 -K 5.0

A CONVENIENT METHOD FOR THE EXCAVATION OF GROWING TREES IN UNDISTURBED SOIL

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It is often desirable to use, as experimental material, a plant grown in the open under normal field conditions and subsequently dug up and potted. This is particularly true of slow-growing bushes and trees, for the establishment of seedlings in large containers may take years, and the filling of soil into such receptacles invariably results in a substrate dissimilar to undisturbed soil. In horticultural practice, the removal of large plants to new sites with the minimum of disturbance to growth is necessary in many instances.

During research on the water relations of coffee it became desirable to use large plants, several years of age. These could be obtained only in the field, and consequently excavation was unavoidable, for it was not possible to wait for the establishment of seedlings. The method of working described in this paper was evolved, and has proved successful. By it, a growing tree together with about 2 tons of soil has been successfully transported, and only the outbreak of war prevented further excavations. There appears to be no upper limit to the size of the soil block that can be isolated in this manner, save only the size of the vehicle available for transport.

The work here reported was done in a district with a very light, friable, volcanic ash soil, with very few stones. The presence of many stones might well make the use of the method difficult, if not impossible.

The necessary apparatus can be made by anyone with a modicum of manual dexterity, and was, in fact, improvised on an East African plantation, from such materials as were to hand. The uses of the method are not confined to the removal of rooted trees: with appropriate modifications it could be used for the preparation of soil monoliths, for the excavation and transport of blocks of undisturbed soil for the construction of lysimeters, or for a number of similar purposes.

DESCRIPTION OF THE METHOD

In essentials, the method consists of the preparation, by mechanical means, of an accurately cone-shaped pillar of soil, of a known and definite angle; of the fitting, to this pillar, of a metal container designed to have the same angle;

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and of the undercutting and removal of the soil block. Since the container fits the block exactly, and since the lift is *via* the sides of the cone-shaped receptacle, no soil disturbance is possible.

The method, in detail, is as follows:

From prior knowledge of the type and extent of the root system and of the amount of root mutilation tolerable, the dimensions of the soil block are determined. For our conditions a truncated cone 120 cm. in diameter at the surface, 90 cm. deep, and with sides inclined 7° from the vertical, proved suitable. A container for the soil block is cut from sheet metal, of thickness appropriate to the size and weight of the tree to be removed. We used $\frac{3}{8}$ -inch galvanized sheet. All necessary joints should be strongly riveted, for there is considerable tension in the metal when the tree is suspended in its container. The cone-shaped receptacle is not completed, but is left in the form of a flat sheet, the final joint being completed in the field after the metal has been wrapped round the soil mass. We used a final joint made from angle irons, firmly riveted to the sheet metal in such a manner that the two flanges could be bolted together to complete the cone. For a larger undertaking, or where a number of similar blocks are to be removed, it might be worth while to arrange for the welding or the riveting of the containers into their final form. Whatever method is adopted, however, it is absolutely essential that the finished container should be of exactly the same shape as the soil pillar.

Hooks, which should be formed from long iron bars, are riveted from top to bottom of the container for its suspension. These, and the general shape of the container, are well seen in plate 1.

A circular trench is now dug round the tree, leaving a central column of soil rather larger than the required size. This trench should be only wide enough to work in with comfort, and should be at least twice the depth of the container.

A frame of fairly heavy timbers is now placed on the soil outside this trench, as shown in figure 1. The upper surface of this is planed true, and should be accurately leveled. For the size of tree that we excavated a square frame proved suitable, but for larger ones a polygonal form may prove to be more convenient.

A central pivot, formed from a short length of split iron piping, clamped between two wooden blocks, is now attached to the trunk of the tree and kept rigid by wedges or by soil packing between it and the trunk. If a soil block alone is to be removed, a simpler form of central pivot can be improvised.

A rider, as illustrated in figure 1, is placed in position on the framework, and leveled by adjusting the height of the central pivot. It will be seen that, although this rider can rotate round the tree trunk, it is located in all other senses by its two long arms which bear on the framework. Thus it is level, and its sides are vertical, in all positions. The rider should be built from sound timber, of such dimensions that it is rigid under normal working stresses. In figures 1 and 2, plate 1, the frame can be seen, and in the latter the rider is shown in position.

The cutter is illustrated in figure 2. It consists of a heavy wooden framework that can rest on the rider, *CD* and *CE* of figure 2 lying along *AB* and

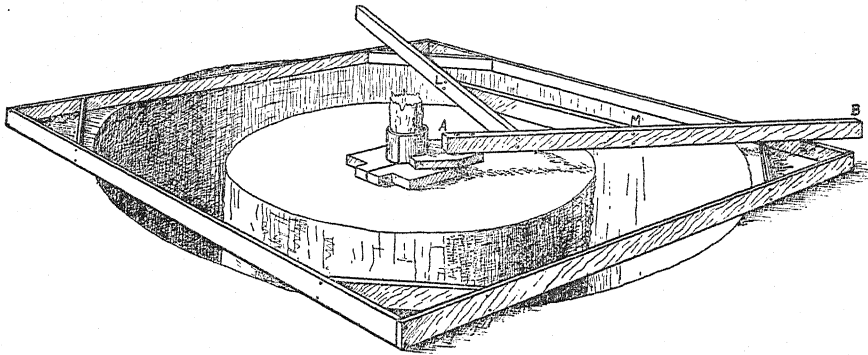


FIG. 1. DIAGRAM OF THE CIRCULAR TRENCH ISOLATING THE SOIL BLOCK TO BE REMOVED, SHOWING THE FRAMEWORK, CENTRAL PIVOT, AND RIDER IN POSITION

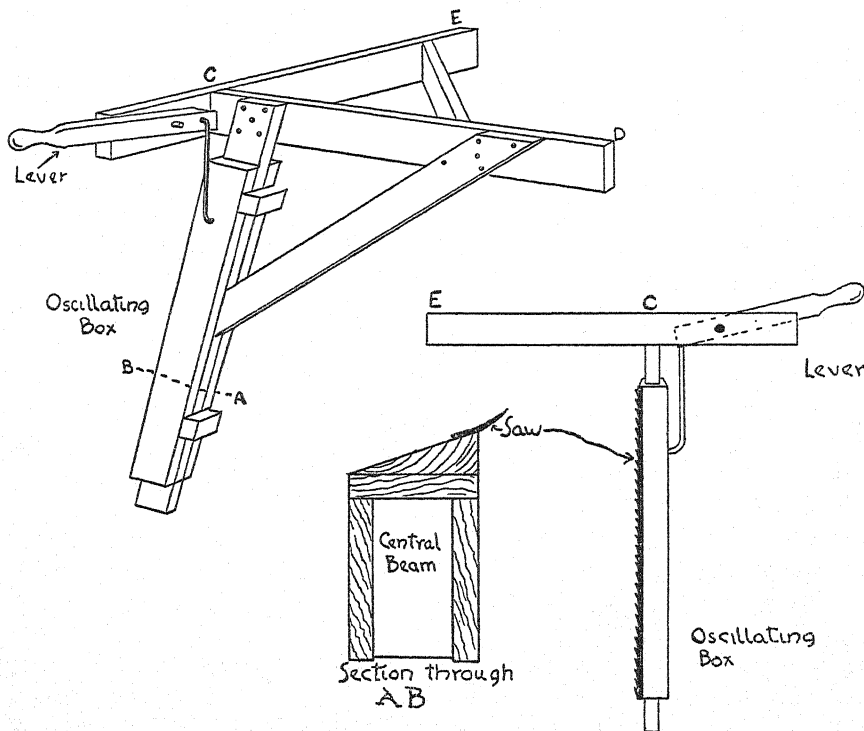


FIG. 2. THE CUTTER

LM respectively of figure 1. Thus any rocking motion is prevented. Dependent from this framework is an arm, fixed at exactly the angle of the container, and suitably braced. This arm should be about one-third longer than

the depth of the container. A closely fitting wooden box slides on it, and can be moved up and down over a few inches by a lever attached to the main framework. This box, on the surface facing the soil pillar, bears a saw blade, set at an angle of about 45° to the direction of cut. This angle is important, for otherwise soil may bind between the back of the blade and the pillar, a clean cut being then impossible.

All the saw teeth should be sharpened to knife-edges, for it is essential that all roots be cut cleanly, and not pulled apart.

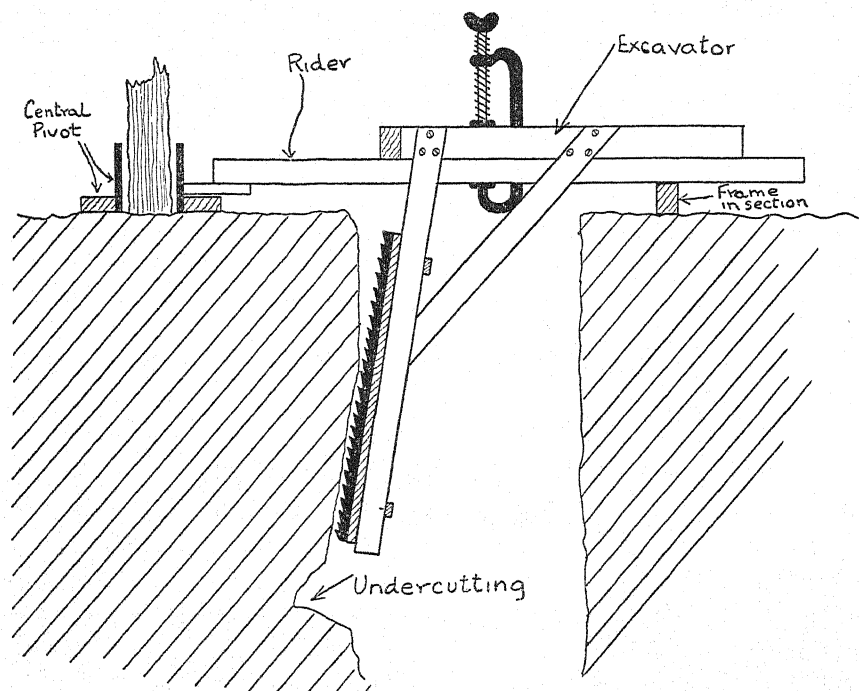


FIG. 3. SECTION THROUGH THE PARTLY COMPLETED TRENCH, SHOWING THE CUTTER IN OPERATION

All moving parts, i.e., the upper surface of the frame, the central pivot, and the inside of the sliding box, are lubricated with soft soap. The cutter is placed in position on the rider, slid into contact with the soil face, and clamped. The rider is then slowly rotated, the saw being operated with the help of the lever all the time. After each rotation, the cutter is moved inward about 1 cm., and the operation is repeated. It is advisable that the bottom of the central pillar be slightly undercut in advance of the saw, as illustrated in figure 3, which shows the central pillar about half-way through the trimming operation. It will be seen that all debris from the saw has free exit, due to the undercutting. The trimming of the pillar is continued until its top is reduced to the diameter of the opening of the container.

The opened container is now lowered into the annular trench and wrapped round the bottom of the soil pillar. As this is of greater depth than the container, and consequently of smaller diameter at the lower end, a loose fit is ensured. The jointing of the container is now completed by any appropriate method.

For the hoisting and transport of the tree we used a crude derrick attached to a 3-ton truck. Figures 2 and 3, plate 1, illustrate the arrangement adopted. Where a "breakdown" van, as used by garages, is available, such a vehicle would be more suitable. From the derrick is hung a small chain-driven pulley-block. The container is attached to this by wires, a spreader (visible in figures 2 and 3, plate 1) ensuring that the direction of pull does not distort the container. When the latter is pulled upward the soil block should exactly fill it, and, with moderate tension in the supporting wires, undercutting can proceed. Inasmuch as the soil in the container is under slight horizontal compression, this presents no difficulties.

After undercutting, the bottom of the container is placed in position on the leveled bottom of the trench, the filled container is lowered on it, and any necessary joints are completed. The whole is then hoisted clear of the ground, as in figure 2, plate 1, and can be driven, slowly, anywhere. For long journeys the container could be lowered on a second truck and driven at normal speeds.

DISCUSSION

The apparatus, as has been mentioned, was improvised in East Africa for field work; money, time, and facilities for manufacture were strictly limited. It could obviously be greatly improved. The substitution of a small metal bandsaw, worked by a crank or a small motor, for the oscillating saw illustrated, and metal construction of the rider and cutter would be an improvement, as would a screw advance for the latter.

SUMMARY

A method, using simple and cheap equipment, is described whereby blocks of undisturbed soil of any reasonable size can be excavated and removed intact.

PLATE 1

EXCAVATION AND TRANSPORT OF A GROWING TREE IN UNDISTURBED SOIL

FIG. 1. Tree before excavation, showing framework and rider in position, cutter and container in background.

FIG. 2. Tree, in container and with base in position, being hoisted from pit.

FIG. 3. Transport of the tree. The native in foreground is controlling oscillation by wire.



FIG. 1



FIG. 2

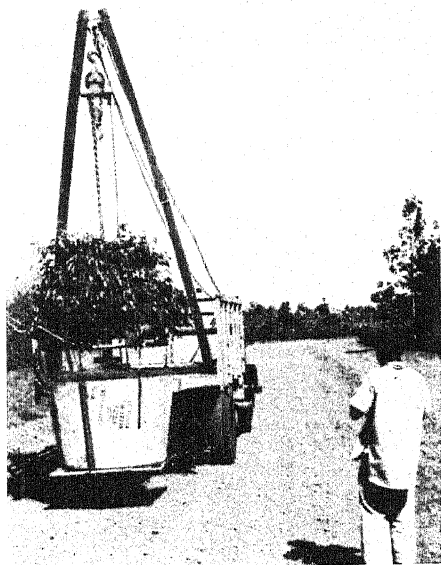


FIG. 3

ASSOCIATION OF LEGUMES AND NONLEGUMES

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Mixed cultivation of legumes and nonlegumes has been practiced in India for ages past. The advantages, as understood by an ordinary farmer, are: first, the better growth of the associated nonlegumes on poor soils, and, second, the prevention of utter failure of the crop in regions of uncertain rainfall. Though the general conceptions may be true, the mode of the associative relationship of the legume and the nonlegume in such mixed croppings is little understood. Recently an impetus to this problem has been given by the extensive researches of Virtanen and his associates.

In 1912, Lipman (1) studied the problem of the associative growth of legumes and nonlegumes. In 20 out of 26 cases reported by him no visible beneficial effect of the legume on the growth of the nonlegume was noticed. His work, however, lay buried until Stallings (4), in 1926, published his positive findings about the beneficial effect of the growth of legumes on nonlegumes. Later, Virtanen and his associates (7, 8, 9, 10), by their extensive work, showed that regular excretions of nitrogen take place from the roots of legumes and that the nonlegume benefits both in growth and in nitrogen content by its association. Though Thornton and Nicol (6) and Nowotnáwna (3) have confirmed Virtanen's findings, a number of other workers, like Wilson (11), Ludwig and Allison (2), and Strong and Trumble (5), have obtained either negative results on the excretion of nitrogen from the legume roots or such contradictory findings that they are inclined to ascribe the phenomenon to a possible disturbance in the physiology of the plants by variations in the substrate or in the photosynthetic activity.

The work undertaken by us has been prompted primarily by the economic importance of the problem in relation to the commonly grown combinations of legumes and nonlegumes in the Punjab. In an attempt to throw some light on the nature of the associative relationship, a number of controlled experiments, in addition to those in soil under natural conditions, have been conducted.

EXPERIMENTAL

In all the experiments reported in this paper, the seeds were sterilized with mercuric chloride before being planted, and those of legumes were later inoculated with pure cultures of the specific strains of nodule bacteria.

In the sand culture experiments, the glasses containing washed and baked sand and adequate CaCO_3 were sterilized for 3 hours in the autoclave at 20 pounds' pressure, and were watered when necessary with sterilized modified Hiltner's nitrogen-free nutrient solution of the following composition: $\text{Ca}_3(\text{PO}_4)_2$, 0.25 gm.; $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, 0.25 gm.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.394 gm.; KCl, 0.25 gm.; FeCl_3 , trace; water, 1000 cc. The containers, in every case, were kept on a stand in a wire-gauze cage.

The experiments in soil were conducted in earthenware pots, each containing 24 pounds of a light loam and watered when necessary with nitrogen-free tap water.

Wheat-chick-pea association

It is a very common practice in the Punjab in regions of uncertain rainfall to sow wheat and chick-peas together. Usually the crop is removed while yet green and is used as cattle fodder.

TABLE 1
Influence of associative growth on yields and nitrogen contents of wheat and chick-pea plants—experiment 1

BEAKER NUMBER	CROP	AVERAGE HEIGHT OF TOPS	AVERAGE NUMBER OF SEEDS PER PLANT	WEIGHT OF CROP*	TOTAL NITROGEN IN CROP	
		cm.		gm.	per cent*	mgm.
1-3	Wheat	36	2.7	1.4	0.558	7.812
4-6	Wheat	39	2.1	1.46	0.67	9.78
	+					
	Chick-pea	25	2.0	2.70	1.43	38.61
7-9	Chick-pea	31	3.0	3.64	1.516	55.182

* Oven-dry basis.

Sand culture experiments. In the first experiment, in nine 600-cc. beakers filled with 800 gm. of sand containing 2 gm. of CaCO_3 , healthy wheat and chick-pea seeds of equal weight were planted as follows: 3 beakers, 2 wheat; 3 beakers, 2 wheat and 2 chick-pea; and 3 beakers, 2 chick-pea. The plants were removed after 5 months, and the yields and nitrogen contents were recorded (table 1). The results show that chick-peas suffer in plant growth as well as in nitrogen content by their association with wheat. Wheat, on the other hand, seems to have benefited slightly.

To determine whether the injurious effect on the growth of chick-peas is proportional to the numbers of the associated wheat plants or, conversely, if wheat derives any benefit from its growth with chick-peas, to find out how many wheat plants can be supported by a single chick-pea plant, a second

experiment was conducted in which eight beakers, each containing 1000 gm. sand, were planted as follows:

<i>Beaker Number</i>	<i>Plants</i>
1-2	1 chick-pea + 1 wheat
3-4	1 chick-pea + 2 wheat
5-6	1 chick-pea + 3 wheat
7-8	1 chick-pea + 4 wheat

The plants were removed at maturity, after 5 months' growth, and the dry weights of plant material and amounts of nitrogen fixed were recorded (table 2).

TABLE 2

Influence of different ratios of chick-pea to wheat plants in associative growth on yields and on nitrogen fixed—experiment 2

BEAKER NUMBER	DRY WEIGHT OF PLANT MATERIAL		WEIGHT OF TOTAL CROP*	TOTAL NITROGEN IN CROP		TOTAL NITROGEN IN SEEDS PLANTED†	TOTAL NITROGEN FIXED
	Wheat	Chick-pea					
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>per cent*</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
1-2	0.45	1.34	1.71	0.97	16.49	11.94	4.55
3-4	0.90	1.7	2.47	1.05	25.93	13.40	12.53
5-6	1.47	1.32	2.64	1.00	26.4	14.86	11.54
7-8	1.62	1.72	3.19	0.91	29.0	16.32	12.68

* Oven-dry basis.

† 1 chick-pea seed = 5.24 mgm. N, 1 wheat seed = 0.73 mgm. N.

The number of seeds per pot were counted, and the amount of nitrogen in the substrate was determined. The results are shown in table 3.

The following conclusions can be drawn as a result of this experiment:

A single chick-pea plant can supply enough nitrogen to support as many as four wheat plants.

The amount of nitrogen fixed by a legume is greater the larger the number of associated nonlegumes (within observed limits). The relative efficiency is greatest when the ratio of chick-peas to wheat is 1:2.

The increase of nitrogen in the sand substrate is also proportional to the number of wheat plants associated with a single chick-pea plant.

The yield of wheat grain per plant suffers when the ratio is 1 chick-pea to 4 wheat. Here it seems that some sort of competition exists between plants to convert the nitrogen into protein reserve.

To determine whether the observed deleterious effect on the growth of chick-peas is in the nature of a crowding-out effect, a third experiment was run in which twenty-seven 1000-cc. beakers filled with 1000 gm. sand were planted to wheat and chick-pea seedlings as shown in table 4. After about 2 months, the chick-pea plants grown alone were observed to be much healthier, greener, and bigger than those grown in association with wheat. Some idea

of the relative plant growth can be obtained from plate 1, figure 1. The results given in table 4 show that chick-peas lose 35 to 40 per cent in size of tops and 14 to 19 per cent in weight by their association with wheat. Wheat, on the

TABLE 3

Influence of different ratios of chick-pea to wheat plants in associative growth on seed production and nitrogen content of the substrate—experiment 2

BEAKER NUMBER	NUMBER OF SEEDS PRODUCED		NITROGEN IN SUBSTRATE*		
	Chick-pea	Wheat	Initial	Final	Increase
			mgm.	mgm.	mgm.
1	4	1	3.5	4.76	1.26
2	2	3			
3	2	4	3.5	7.56	4.06
4	4	5			
5	3	7	3.5	7.80	4.30
6	2	6			
7	4	6	3.5	8.68	5.18
8	6	3			

* Per 100 gm.

TABLE 4

Influence of associative growth of chick-pea and wheat on the relative size and yield of plants—experiment 3

Yields on dry-weight basis

BEAKER NUMBER	PLANTS	CHICK-PEA		WHEAT		CHICK-PEA, PER CENT GAIN OR LOSS OVER CONTROL		WHEAT, PER CENT GAIN OR LOSS OVER CONTROL	
		Average height of tops	Average yield per beaker	Average height of tops	Average yield per beaker	Height	Weight	Height	Weight
		cm.	gm.	cm.	gm.				
1-3	1 wheat + 1 chick-pea	12	0.25	26	0.348	-40	-14	-16.1	+11.4
4-6	2 wheat + 2 chick-pea	12	0.432	22	0.415	-36.8	-13.6	-12.0	-10.0
7-9	3 wheat + 3 chick-pea	15	0.56	22	0.486	-34.8	-18.6	0	-19.0
10-12	1 wheat	31	0.312
13-15	2 wheat	25	0.46
16-18	3 wheat	22	0.59
19-21	1 chick-pea	20	0.29
22-24	2 chick-pea	19	0.50
25-27	3 chick-pea	23	0.68

other hand, does not obtain a corresponding benefit in all cases. In a combination of three wheat and three chick-pea plants a slight crowding-out effect due to the limited surface of the beaker is noticeable.

A fourth carefully controlled experiment was conducted to determine whether nitrogen is actually excreted from the roots of chick-peas. The bottoms of four Winchester bottles were knocked off and the edges ground. Each bottle was then inverted and fitted with a wooden stopper, through which

TABLE 5

Nitrogen, in milligrams, excreted from roots of chick-pea alone and in associative growth with wheat—experiment 4

NITROGEN	BOTTLE 1— 3 CHICK-PEA	BOTTLE 2— 3 CHICK-PEA + 3 WHEAT	BOTTLE 3— 6 CHICK-PEA	BOTTLE 4— 3 CHICK-PEA + 6 WHEAT
<i>After 28 days</i>				
Organic.....	0.35	0.35	0.28	0.35
Nitrite.....	0.028	0.011	0.039	0.012
Nitrate.....	0.156	0	0.206	0
Total.....	0.534	0.361	0.525	0.362
<i>After 42 days</i>				
Organic.....	0.42	0.14	0.28	0
Nitrite.....	0.003	0.001
Nitrate.....	0	0	0	0
Total.....	0.423	0.14	0.281	0
<i>After 60 days</i>				
Organic.....	0.28	0.28	0.28	0.21
Nitrite.....	0.003	0.004
Nitrate.....	0	0	0	0
Total.....	0.283	0.28	0.284	0.21
<i>After 90 days</i>				
Organic.....	0.56	0.42	0.42	0.42
Nitrite.....	0.003	0.002	0.002	0.003
Nitrate.....	0	0	0	0
Total.....	0.563	0.422	0.422	0.423
<i>After 140 days</i>				
Organic.....	1.4	0.98	1.05	0.91
Nitrite.....	0.005	0.014	0.025	0.019
Nitrate.....	0	0	0	0
Total.....	1.405	0.994	1.075	0.929

a piece of glass tubing passed. The lower end of the glass tube was closed with a rubber tubing and pinchcock. While the bottles were in this position, glass beads were placed at the bottom and covered with glass wool. The bottles were then filled with 2000 gm. of sand (fig. 1) and planted with seedlings as indicated in table 5.

At the end of 4 weeks and at frequent intervals thereafter, the roots of the plants in each bottle were washed with sterilized distilled water by suction, and the amounts of organic, nitrite, and nitrate nitrogen were determined in the extract by micromethods. Since the amount of excreted nitrogen was very small, it was not possible to conduct a qualitative test for amino acids.

The results, recorded in table 5, show that washings of legume roots contain traces of nitrogen; the amount of nitrogen excreted is greater when the legume is grown alone than when it is associated with a nonlegume—in the latter case, probably the associated nonlegume takes up the excreted nitrogen; the amount of excretion is not proportional to the numbers of legume plants.

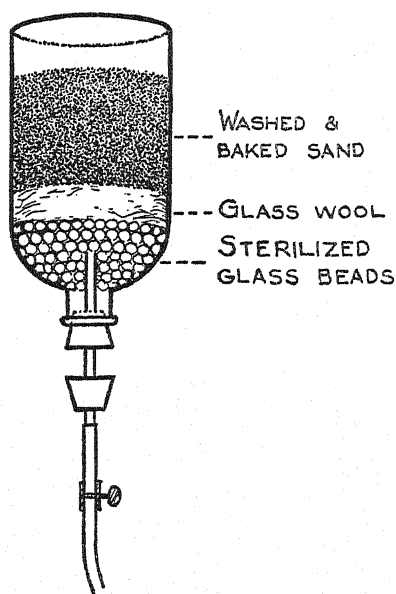


FIG. 1. BOTTLE USED IN THE STUDY OF NITROGEN EXCRETION FROM CHICK-PEA ROOTS

Table 6 shows that the legume definitely suffers in growth while in association with the nonlegume. A duplicate test gave comparable results. The general appearance of the crop is shown in plate 1, figure 2.

Pot experiment in soil. To test whether the foregoing findings, obtained in sand cultures, apply equally well to plant growth in soil under natural conditions, an experiment was conducted in which 12 pots of soil were sown as follows: 4 pots, 6 wheat; 4 pots, 6 wheat and 6 chick-pea; 4 pots, 6 chick-pea.

After the plants had grown for about a month, microscope slides were embedded for 48 hours near the roots in different pots. They were stained with phenolic rose bengal according to Cholodny's method, and on examination revealed that there was a much greater variety of microorganisms in pots containing both legumes and nonlegumes than in pots containing either alone. There was, however, a larger number of microorganisms on slides from the legume pots than on those from the nonlegume pots.

The crop was removed at maturity, and the yield and nitrogen content of the plant material were recorded (table 7). The results clearly show that the chick-pea plant suffers immensely in crop growth as well as in nitrogen content by its association with wheat; wheat, on the other hand, gains slightly in relative nitrogen content but loses in plant growth. The general appearance of the plants after 3 months is shown in plate 1, figure 3.

TABLE 6
Influence of associative growth on yields of chick-pea and wheat—experiment 4

BOTTLE NUMBER	CHICK-PEA		WHEAT	
	Pods and seeds	Total dry weight <i>gm.</i>	Ears and grains	Total dry weight <i>gm.</i>
1	2 pods 2 seeds	1.21
2	1 pod 1 seed	0.75	3 ears 3 grains	0.965
3	4 pods 4 seeds	1.97
4	2 pods 1 seed	0.77	4 ears 5 grains	1.315

TABLE 7
Influence of associative growth on yield and nitrogen content of chick-pea and wheat grown in soil

POT NUMBER	CROP	DRY WEIGHT			NITROGEN CONTENT			LOSS OF NITROGEN BY CROP ASSOCIATION
		Grain <i>gm.</i>	Straw <i>gm.</i>	Total crop <i>gm.</i>	Grain <i>per cent</i>	Straw <i>per cent</i>	Total crop <i>mgm.</i>	
1-4	Wheat	29.7	43.8	73.5	1.618	0.497	698.22
5-8	Wheat +	20.0	32.0	52.0	1.618	0.577	506.0	-27.5
	Chick-pea	18.5	30.5	49.0	3.335	0.742	836.0	-55.3
9-12	Chick-pea	41.0	63.0	104.0	3.335	0.80	1871.0

Senji-oat association

Sand culture experiment. To study the associative relationship of senji (*Melilotus parviflora*) and oats, a controlled experiment was run in beakers containing sand as follows: beakers 1-9 and 19-27, 1000 gm. each; beakers 10-18, 800 gm. each. Senji and oat seeds were germinated in sterilized petri dishes, and seedlings were planted as shown in table 8. Because of their slow growth, the senji seedlings were planted about a month earlier than the oat seedlings. The results of observations on the height of tops (pl. 1, fig. 4), yield, and nitrogen content in different cases are analyzed in table 8. It will

be seen from these data that senji loses over 60 per cent in the height of tops and 86-88 per cent in total dry weight when it is associated with oats. Whether the oats derive a corresponding benefit from their association with senji is not definitely established. It is hard to decipher any crowding-out effect. No significant difference is apparent in the nitrogen content of either oats or senji whether grown alone or in association.

TABLE 8

Influence of associative growth of senji and oats on the relative size, yield, and nitrogen content of plants

Yields and nitrogen content on dry-weight basis

BEAKER NUMBER	PLANTS	SENJI			OATS			SENJI, PER CENT GAIN OR LOSS OVER CONTROL		OATS, PER CENT GAIN OR LOSS OVER CONTROL	
		Average height of tops	Average yield per beaker	Nitrogen con- tent	Average height of tops	Average yield per beaker	Nitrogen con- tent	Height	Weight	Height	Weight
		cm.	gm.	per cent	cm.	gm.	per cent				
1-3	1 senji + 1 oat	12	0.16	2.4	37	0.75	0.64	-66	-88.2	-20	-38.0
4-6	2 senji + 2 oat	15	0.32	2.46	37	1.365	0.56	-64.3	-87.0	+15	+25.2
7-9	3 senji + 3 oat	14	0.442	2.10	30	1.79	0.55	-62.1	-86.7	0	-1.6
10-12	1 oat	47	1.21	0.74
13-15	2 oat	32	1.09	0.60
16-18	3 oat	30	1.82	0.63
19-21	1 senji	54	1.36	2.52
22-24	2 senji	42	2.42	2.42
25-27	3 senji	37	3.305	2.18

TABLE 9

Influence of associative growth on size, yield, and nitrogen content of senji and oats grown in soil

Yields on air-dry basis

POT NUMBER	CROP	OATS		SENJI		HEIGHT OF PLANTS	WEIGHT OF ROOTS
		Yield of tops	Nitrogen content	Yield of tops	Nitrogen content		
		gm.	per cent	gm.	per cent		
1-3	Oats alone	50.25	1.91	8.98
4-6	Oats + senji	56.73	1.60	4.76	2.14	15-46	8.50
7-9	Senji alone	36.5*	2.88	65-76	2.6*

* White ant damage.

Pot experiment in soil. Nine pots of soil were seeded to oats and senji as follows: 3 pots, 6 oat; 3 pots, 6 oat and 6 senji; 3 pots, 6 senji. There was an astonishingly great difference between the growth of senji plants alone and in association with oats. Plate 1, figure 5, shows the stunted growth of those grown in association with oats.

The plants were removed after 5 months' growth, and the sizes, yields, and nitrogen content were recorded (table 9). The results conclusively show that senji suffers immensely in growth and in nitrogen content of the crop material by its association with oats. The oats, on the other hand, gain a little in plant growth.

Chari-guara association

It is a very common practice in the Punjab to sow chari (*Andropogon sorghum*) and guara (*Cyamopsis psoraloides*) in association during the hot weather for fodder purposes. The interrelationship between the two was studied by means of an experiment conducted with 21 pots of soil planted as shown in table 10. In general, chari plants when associated with guara presented a much better appearance than when grown alone. The growth of guara, on the other hand, was much poorer where it was associated with chari.

TABLE 10
Influence of chari-guara association on size and weight of plants
Yields on green-weight basis

POT NUMBERS	CROP RATIO	CHARI		GUARA		CHARI, PER CENT GAIN OR LOSS OVER CONTROL		GUARA, PER CENT GAIN OR LOSS OVER CONTROL	
		Average height per plant	Average weight per plant	Average height per plant	Average weight per plant	Height	Weight	Height	Weight
		in.	gm.	in.	gm.				
1-3	4 chari each	37½	19.25
4-6	4 guara each	20	29.0
7-9, 13-17	4 chari:4 guara	34	17.5	13	9.5	-9.3	-9.9	-35	-67.2
10-12	2 chari:2 guara	43	31.5	16	16.8	+14.7	+63.6	-20	-42.0
18-21	3 chari:3 guara	43	35.7	14½	10.7	+14.7	+85.5	-27.5	-63.1

After growth had continued for about 3 months, the size of the tops was measured, and the plants were removed and weighed. The results, recorded in table 10, show that guara suffered immensely in its association with chari, a loss in height of 20 to 35 per cent and in green weight of 42 to 67 per cent having been observed; chari gained in height and in green weight when the ratio of chari to guara was 2:2 and 3:3, but not when the ratio was 4:4, apparently because of a crowding-out effect in small pots.

DISCUSSION

Though the precautions observed to prevent contamination of the seed and substrate in all sand culture experiments do not rule out the possibility of later contaminations having occurred, this seems to be of no great significance, particularly as no attempts have been made to define the type of nitrogen excreted in different cases.

A definitely injurious effect on the growth of the legumes tested, viz., chick-pea, senji, and guara has been recorded in all cases. Of the associated non-legumes, wheat and oats have shown little, if any, gain in crop weight or nitrogen content as a result of their association with chick-pea and senji respectively. Chari, on the other hand, has shown a considerable gain both in height and in weight of plants as a result of its association with guara. It is reasonable, therefore, to infer that in certain combinations the nonlegume does benefit by its association with the legume, while in others it does not. But why it does in certain cases and not in others might be traced to the beneficial effect of a legume or to the receptive capacity of a nonlegume. It is interesting to record that in a nitrogen-deficient medium the legume can be made, within limits, to fix more nitrogen by its association with the nonlegumes.

The excretions of nitrogen from the roots of a legume grown alone or in association with a nonlegume have been recorded, but the injury to the legume cannot be entirely explained on the basis of the uptake of excreted nitrogen by the associated nonlegume. Had this been so, the nonlegume should have shown better growth in all associated cultures, but it did not. It is possible that in certain cases the very presence of roots of the nonlegume in proximity to those of the legume exerts some deleterious effect on the uptake of fixed nitrogen by the latter. The exact nature of this influence will form the subject of a further study.

SUMMARY

Chick-pea suffers both in growth and nitrogen content by its association with wheat. The loss of chick-pea may amount to as much as 35-40 per cent in size and 14-20 per cent in weight.

A single chick-pea plant can support the growth of as many as four wheat plants. The relative efficiency of the nitrogen fixation process appears to be greatest when the ratio of chick-pea to wheat is 1:2.

The amount of excreted nitrogen in the substrate is also greater the larger the number of wheat plants associated with a single chick-pea plant, within observed limits.

The grain formation in wheat seems to suffer where the ratio of chick-pea to wheat is 1:4.

Definite excretions of nitrogen were recorded from the roots of chick-pea. Since the amount of excretion was very small it was not possible to make a qualitative test for amino acids, but nitrites were generally found to be present.

The beneficial effect of the association of chick-pea on the growth of wheat is not marked or constant.

Senji loses 60 per cent in height of plants and about 88 per cent in total dry weight by its association with oats. Oats do not gain significantly by their association with senji.

Guara suffers immensely in growth by its association with chari. Chari gains greatly by its association with guara.

REFERENCES

- (1) LIPMAN, J. G. 1912 Associative growth of legumes and nonlegumes. *N. J. Agr. Exp. Sta. Bul.* 253.
- (2) LUDWIG, C. A., AND ALLISON, F. E. 1937 Experiments concerning diffusion of nitrogenous compounds from healthy legume nodules or roots. *Bot. Gaz.* 98: 680-695.
- (3) NOWOTNÓWNA, A. 1937 Nitrogen uptake in mixed crops not receiving nitrogenous manure. *Jour. Agr. Sci.* 27: 503-510.
- (4) STALLINGS, J. H. 1926 Form of legume nitrogen assimilated by nonlegumes when grown in association. *Soil Sci.* 21: 253-276.
- (5) STRONG, T. H., AND TRUMBLE, H. C. 1939 Excretion of nitrogen by leguminous plants. *Nature* 143: 286-287.
- (6) THORNTON, H. G., AND NICOL, H. 1934 Further evidence upon the nitrogen uptake of grass grown with lucerne. *Jour. Agr. Sci.* 24: 540-543.
- (7) VIRTANEN, A. I., AND HAUSEN, S. VON 1931 Studies on leguminous bacteria and plants: IX. *Biochem. Ztschr.* 232: 1-14.
- (8) VIRTANEN, A. I., AND HAUSEN, S. VON 1935 Root nodule bacteria of leguminous plants: XVI. *Jour. Agr. Sci.* 25: 278-289.
- (9) VIRTANEN, A. I., HAUSEN, S. VON, AND KARSTRÖM, H. 1933 Legume bacteria and plants: XII. *Biochem. Ztschr.* 258: 106-117.
- (10) VIRTANEN, A. I., HAUSEN, S. VON, AND LAINE, T. 1937 Root-nodule bacteria of leguminous plants: XIX, XX. *Jour. Agr. Sci.* 27: 332-348, 584-610.
- (11) WILSON, P. W. 1937 Excretion of nitrogen by leguminous plants. *Nature* 140: 154-155.

PLATE 1

ASSOCIATIVE GROWTH OF CHICK-PEAS AND WHEAT AND OF SENJI AND OATS

FIG. 1. Left to right—1 chick-pea + 1 wheat, 1 chick-pea, 2 chick-pea, 3 chick-pea, 2 chick-pea + 2 wheat, 3 chick-pea + 3 wheat, 1 wheat, 2 wheat, 3 wheat plants

FIG. 2. Left to right—3 chick-pea, 3 chick-pea + 3 wheat, 6 chick-pea, 3 chick-pea + 6 wheat plants

FIG. 3. Left to right—6 chick-pea, 6 wheat + 6 chick-pea plants

FIG. 4. Left to right—1 senji + 1 oat, 1 senji, 2 senji, 3 senji, 2 senji + 2 oat, 3 senji + 3 oat plants

FIG. 5. Left to right—6 senji, 6 senji + 6 oat plants

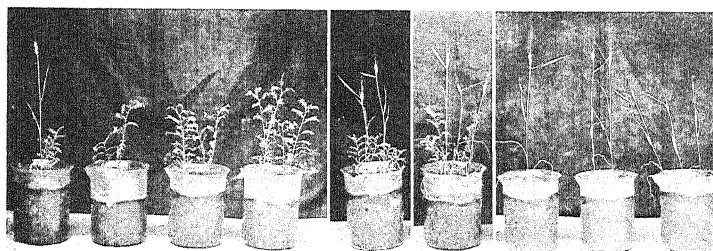


FIG. 1

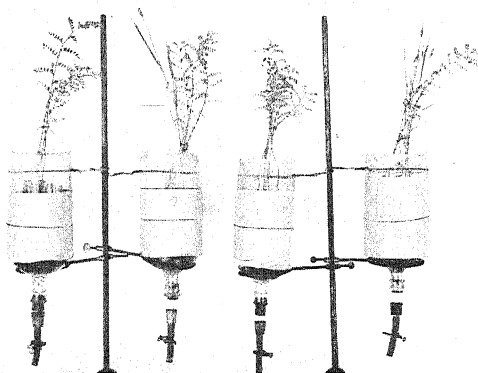


FIG. 2

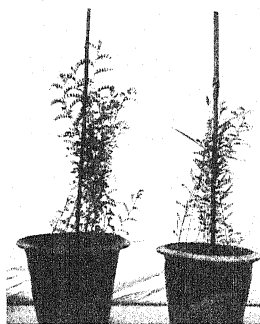


FIG. 3

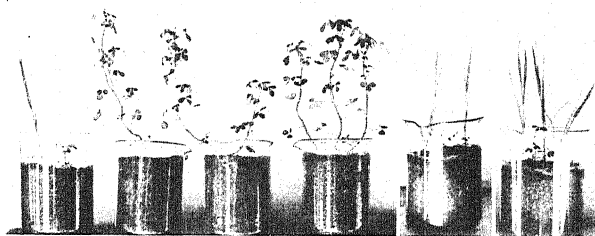


FIG. 4



FIG. 5

A STUDY ON THE CHEMICAL NATURE OF HUMIC ACID¹

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A certain portion of the heterogeneous soil organic matter is apparently more or less resistant to rapid decomposition and remains for a time in the soil, or may even accumulate in the soil, under certain conditions. This dark brown to black fraction is often known as "humus." As used in this paper, however, the term "humus" refers to that portion of the soil organic matter peptized by 4 per cent ammonia. That fraction peptized by 4 per cent ammonia, precipitated by strong acids, and insoluble in 95 per cent ethanol, is designated as "humic acid." It has been variously designated as "black pigment" (2), "humus acid," "sacculmic acid," "mull acid," "sucrohumic acid," and "lignohumic acid." Although a vast amount of research has been directed along this line, and our knowledge about soil organic matter has increased tremendously, final solution as to the exact nature of humic acid has not been forthcoming. Early researches usually consisted of extraction of soil humus by dilute alkali, followed by attempted fractionation of the isolated product by means of various solvents. The fractions obtained were amorphous, colloidal, and of indefinite composition. They were given various names, which had no real chemical significance and tended only to confuse the study of soil organic matter. Many theories have been propounded to explain the formation of humic acid, but the most widely accepted one was advanced by Waksman (13), who holds that lignin combines with a proteinlike material, under the influence of microorganisms, to form humic acid.

The colloidal organic fraction is one of the most active components of the soil, and it is evident that a knowledge of humic acid is essential if we are to understand the origin and development of soils, as well as certain processes that influence plant growth. Since very little is definitely known about the chemical nature of this organic complex and since most of the early research work was performed on the humic acid fraction isolated from peats, it was the object of this investigation to isolate the humic acid from prairie, muck, and forest soils, and to study each acid in detail. In this way the similarities or differences between the acids could be pointed out, a few definite physical and

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chemical properties could be determined, and these in turn might lead to some generalizations concerning the chemical constitution and the method of formation of the humic acid.

EXPERIMENTAL

Extraction of the humic acids

Approximately 3 kgm. of a well-decomposed, cultivated muck soil from Minnesota was leached with 1 per cent hydrochloric acid to remove all traces of soluble calcium. The muck was then transferred to a carboy and the humus extracted by 4 per cent ammonia. After the muck had been subjected to extraction by intermittent shaking for 24 hours, the suspension was allowed to settle for 2 or 3 days. The jet-black supernatant liquid was siphoned off and passed through a Sharples centrifuge, the cylinder of which had been lined with a tight-fitting sheet of celluloid. Virtually all the clay remaining in suspension was thrown out on the celluloid sheet and discarded. Four per cent ammonia was again added to the muck which remained in the bottle, and the extraction was repeated.

The centrifuged suspension was acidified with hydrochloric acid, and the jet-black gelatinous precipitate was allowed to settle. The clear supernatant liquid was then siphoned off and discarded, and the remaining black colloidal suspension was passed through the Sharples centrifuge. The humic acid fraction was thus thrown out on the celluloid sheet. It was dried at 70°C., pulverized in a glass mortar, copiously washed with water, leached with 95 per cent ethanol, and again washed thoroughly with water. It was then dialyzed in cellophane and finally was air-dried and labeled P2. This is the so-called humic acid. It contained 7.77 per cent ash on the oven-dry basis.

The filtrate after precipitation and removal of the humic acid usually showed the presence of organic indicators. When acid, the filtrate was pale yellow; and when alkaline, it had a distinct greenish tint.

Humic acid was extracted from the gray-brown forest soils of Michigan merely by leaching several kilograms of the soil with a solution consisting of 4 per cent ammonia and 2 per cent ammonium carbonate, the soils having been previously leached with 1 per cent hydrochloric acid to the absence of calcium in the leachate. The resultant black solution was concentrated and then acidified with hydrochloric acid, which caused the precipitation of the black flocculent humic acid fraction. The suspension was transferred to a carboy and allowed to settle for 2 days. The clear supernatant liquid was then siphoned off, and distilled water was added while the mixture was agitated. This was again allowed to settle for several days, and the process was repeated. The humic acid fraction was washed eight to twelve times in this manner and then dialyzed in cellophane. Finally, it was transferred to a large evaporating dish and dried under a radiant heater. The temperature never exceeded 70°C. The humic acid fraction was ground, washed again with water, leached with 95 per cent ethanol, and finally leached with more

water. The humic acid thus obtained was air-dried and labeled P3. The ash content was 3.60 per cent on the oven-dry basis.

The humic acid from the grassland soils of the western Great Plains area was extracted in exactly the same manner as that used on the gray-brown forest soils. All of the ammoniacal extracts from the 300 samples in the Great Plains area, used in the study of pigment distribution (1), were collected and concentrated. The concentrate was then acidified, and the same procedure as above was used. The dry humic acid fraction was ground, washed with water, leached with 95 per cent ethanol and again washed with water. The humic acid obtained, labeled P1, had an ash content of 6.86 per cent on the oven-dry basis.

The freshly prepared humic acid was very voluminous, as a result of adsorbed water, and when separated from the dispersion medium it formed an elastic gel. It shrank enormously on drying and eventually became a brittle solid. When dry, the acid from all three sources would not again form a

TABLE 1
Analyses of humic acids
In per cent on the oven-dry, ash-free basis

HUMIC ACID	SOURCE	C	H	N	O	ASH
P1	Grassland soils from the Great Plains	58.35	4.54	5.88	31.2	6.86
P2	Minnesota muck soil	58.81	5.54	5.49	30.2	7.77
P3	Michigan forest soils	55.58	5.17	6.03	33.2	3.60

suspension upon addition of water. If kept moist, however, it is a reversible colloid. The humic acid apparently exists in the soil solution as a hydrophilic colloid.

Various methods of purification were tried with little success. Repeated dispersion by ammonia and precipitation by acid caused no appreciable decrease in percentage of ash. One portion of the washed and dried humic acid was dissolved in a relatively small amount of ammonium hydroxide and placed in an electrodialysis cell. The suspension was electrodialyzed for 12 hours using 90 volts and 50 amperes. At the end of the 12 hours the solution was neutral to litmus, and most of the humic acid was coagulated upon the parchment next to the anode. The ash content was reduced only very slightly. A small portion of the humic acid, P2, was purified by reprecipitation and dialysis to such an extent that its ash content was reduced to 1.20 per cent. Suspensions of the humic acids in water, pyridine, sodium hydroxide, ammonium hydroxide, and alcohol gave distinct Tyndall cones. Furthermore, the light reflected from these cones was polarized, conclusive evidence that the acids form colloidal suspensions in the above solvents.

The analyses of the three humic acids are listed in table 1. The nitrogen

content was determined by the Kjeldahl method, and percentages of carbon and hydrogen were determined by dry combustion. The ash content of each acid was determined by igniting in an electric furnace at 700–750°C.

Acetylation

Eight grams of the humic acid were suspended in 40 cc. of acetic anhydride, and 10 drops of concentrated sulfuric acid was added. The suspension was then heated in a water bath at 100°C. for 8 to 10 hours and was stirred by intermittent shaking. In every case the acids were insoluble in the anhydride. The contents of the flask were then emptied into a large volume of ice water, thoroughly mixed, and filtered. The acetylated humic acid was washed until the filtrate was neutral to bromthymol blue. It was then air-dried, ground, and percentage ash and moisture determined. In one instance dry hydrogen chloride gas was introduced into the flask in place of the sulfuric acid. Subsequent analysis showed no difference in acetyl content between this product and that obtained by using sulfuric acid.

Humic acids P1, P2, and P3 were acetylated readily even in the cold. Upon addition of acetic anhydride and sulfuric acid an exothermic reaction took place. The reaction with P2 was apparently the most active, since the reaction flask became hot, whereas with P1 and P3 the reaction flasks became warm. The following analysis shows the relative amount of acetylation that took place at room temperature:

HUMIC ACID	PER CENT ACETIC ACID	ACETYLATED PRODUCT	PER CENT ACETIC ACID
P1	1.33	Ac1	1.76
P2	1.00	Ac2	5.62
P3	1.50	Ac3	2.48

The acetylated products were just as insoluble as the original humic acids, being peptized only by dilute alkalies.

Determination of acetyl content

The method employed in determining acetyl content, with slight modification, was that of Perkins (9). The sample, in 0.5 to 1.0 gm. quantity, along with 25 cc. of 95 per cent aldehyde-free ethanol was placed in a round-bottom flask, connected to a condenser. Five cubic centimeters of concentrated sulfuric acid was added dropwise, and the mixture was refluxed for 15 minutes. The water in the condenser was then drained, and the acetic acid was swept, by a slow stream of alcohol vapor, out of the reaction flask into an Erlenmeyer containing 25 cc. of 0.2 *N* alcoholic potassium hydroxide. This procedure was continued until the volume of the distillate in the receiving flask was approximately 150 cc. During the reaction, which required about an hour for completion, the water bath around the reaction flask was heated at such a rate that the contents of the flask were reduced in volume about one half. The distil-

late was then refluxed for 30 minutes, diluted with distilled water, and the unused potassium hydroxide determined by titration with 0.1 *N* hydrochloric acid.

The results are presented in table 2, expressed as percentage of acetic acid and also as milliequivalents of acetic acid per 100 gm. of water-free, ash-free sample.

The acetyl content of all three humic acids is relatively low, and their behavior toward acetylation is very similar to that of lignin isolated from soil and from corncobs by alcoholic NaOH.

TABLE 2
Acetyl content of certain organic complexes

SAMPLE	SOURCE	ACETYL CONTENT	
		Per cent acetic acid	M.e. of acetic acid per 100 gm. of sample
P1.....	Grassland soils from the Great Plains	1.33	22
P2.....	Minnesota muck soil	1.00	16
P3.....	Michigan forest soils	1.50	25
Ac1.....	Acetylated humic acid P1	12.36	206
Ac2.....	Acetylated humic acid P2	18.79	313
Ac3.....	Acetylated humic acid P3	18.53	308
Lignin 15*.....	Marshall clay loam	1.5	25
Ac 15*.....	Acetylated lignin 15	22.9	381
Lignin 6*.....	Corncobs	0.2	4
Ac 6*.....	Acetylated lignin 6	13.6	227
Lignin†.....	Corncobs	24.13	404
Lignin†.....	Oat hulls	23.44	392

* Data from Weldon (14).

† Data from Phillips (10).

Ac = acetylated.

Methylation

Humic acids P1, P2, and P3 were methylated by suspending a 5-gm. sample in 100 cc. of water containing 10 cc. of a 50 per cent solution of potassium hydroxide. The mixture was agitated by a mechanical stirrer, and 20 cc. of dimethyl sulfate was added dropwise. From 10 to 20 cc. more of potassium hydroxide was needed in each case to keep the suspension alkaline during the course of the reaction. The reaction is exothermic; consequently, to prevent overheating, the flask was surrounded by cold water at the start of the reaction. Stirring was continued from 1 to 2 hours at room temperature. The flask was then gradually warmed to 60°C. in a water bath and held at this temperature

for 2 hours. After standing overnight, the suspension was poured into a large volume of cold water containing a few drops of sulfuric acid. The mixture was agitated and filtered. Since no alkali-insoluble product could be separated, all of the humic acid was precipitated whether methylated or not. The black gelatinous precipitate was dried at 60°C., ground, and washed with water. The sample was then returned to the methylating flask, and the above procedure was repeated. Each sample was methylated three times, or until the methoxyl content became constant. The final product was then filtered, dried, and ground. The dry sample was washed with distilled water, 50 per cent alcohol, and finally leached again with copious amounts of water. The behavior of the three methylated humic acids toward all solvents was exactly the same as that of the corresponding humic acids.

TABLE 3
Methoxyl content of humic acid, methylated humic acid, and certain lignins

SAMPLE	SOURCE	METHOXYL CONTENT	
		Per cent	M.e. per 100 gm. of sample
P1.....	Grassland soils from the Great Plains	1.03	33
P2.....	Minnesota muck soil	1.67	54
P3.....	Michigan forest soils	1.74	56
Me1.....	Methylated humic acid P1	8.71	281
Me2.....	Methylated humic acid P2	8.98	289
Me3.....	Methylated humic acid P3	7.92	255
Lignin 6*.....	Corn cobs	12.9	444
Lignin 25*.....	Marshall clay loam	1.6	56
Me lignin 6*.....	Corn cobs	27.1	935
Me lignin 25*.....	Marshall clay loam	17.5	603
Lignin†.....	Oat hulls	15.6	503
Me lignin†.....	Oat hulls	31.7	1022

* Data from Weldon (14).

† Data from Phillips (11).

Me = methylated.

Determination of methoxyl content

The method employed in determining methoxyl content was that of Phillips (12). Approximately 0.3 gm. of sample and 10 cc. of hydriodic acid were placed in the apparatus, and the flask was heated to 130–140°C. The condenser was maintained at a temperature of 50–60°C. A slow stream of carbon dioxide carried the methyl iodide out of the reaction flask and into the alcoholic silver nitrate. The silver iodide was filtered, washed, dried, and weighed.

The results, calculated as per cent methoxyl and also as millequivalents of methoxyl per 100 gm. of sample, on the ash-free, moisture-free basis, are presented in table 3.

Base-exchange capacity

Various investigators (4, 6, 7) have shown that organic materials possess base-exchange properties and that the exchange capacity of the organic materials increases as the decomposition progresses. Other workers (3, 7) have concluded that the base-exchange capacity of organic materials is due to certain groups, such as the phenolic hydroxyl and the carboxyl. If this be true, the effect of blocking these groups by acetylation and methylation

TABLE 4
Exchange capacity of humic acid, acetylated and methylated humic acid, and certain lignins

SAMPLE	SOURCE	BASE-EXCHANGE CAPACITY, AVERAGE M.E. OF CALCIUM AND BARIUM PER 100 GM. OF SAMPLE	LOSS OF EXCHANGE CAPACITY, M.E. PER 100 GM. OF SAMPLE
P1.....	Grassland soils from the Great Plains	394	
P2.....	Minnesota muck soil	274	
P3.....	Michigan forest soils	253	
Ligno-humate*.....	Average of 10 soils	382†	
Lignin 6‡.....	Corncocks	28	
Lignin 25‡.....	Marshall clay loam	339	
Ac1.....	Acetylated humic acid P1	342	52
Ac2.....	Acetylated humic acid P2	248	26
Ac3.....	Acetylated humic acid P3	224	29
Ac Lignin 6‡.....	Corncocks	8	20
Ac Lignin 25‡.....	Marshall clay loam	74	265
Me1.....	Methylated humic acid P1	273	121
Me2.....	Methylated humic acid P2	227	47
Me3.....	Methylated humic acid P3	246	7
Me Lignin 6‡.....	Corncocks	2	26
Me Lignin 25‡.....	Marshall clay loam	9	330

* Data from McGeorge (4).

† M.e. of calcium only.

‡ Data from Weldon (14).

can be studied by means of the base-exchange reaction. Therefore the base-exchange capacities of all three humic acids and of their corresponding acetylated and methylated products were determined by the following procedure:

A sample weighing 0.5 to 1.0 gm. was placed in a Gooch crucible, leached with 150 cc. of neutral normal calcium acetate solution, and then washed with CO₂-free water to the absence of calcium in the filtrate. The sample was then leached with 150–175 cc. of 0.1 N hydrochloric acid, and the calcium con-

tent of the filtrate was determined. After leaching with acid, the sample was washed with distilled water, and the procedure was repeated, using neutral normal barium acetate solution. The results, calculated in terms of milliequivalents of exchangeable cation per 100 gm. of water-free, ash-free material, are presented in table 4.

From these data we note that both acetylation and methylation of the humic acids decreased the base-exchange capacity slightly, methylation being more effective than acetylation in blocking off certain groups involved in the base-exchange reaction. The greatest loss in exchange capacity always occurred with humic acid P1. Similarly, we note that the loss in exchange capacity of corncob lignin caused by acetylation and methylation was of the same order as that for P2 and P3. The effect of methylation and acetylation on the exchange capacity of soil lignin, however, is several times as great as the effect on humic acid. In every instance the decrease in exchange capacity is many times less than equivalent to the increase in acetyl or methoxyl content. This appears to indicate the presence of hydroxyl groups that may readily be esterified and methylated but that are not active in the base-exchange reaction. These may be enolic, alcoholic, or very weakly acidic phenolic hydroxyl groups. If it is assumed that the base exchange of the humic acids is due primarily to phenolic hydroxyl groups, some of the hydroxyls must not be esterified by acetic anhydride under the conditions of this investigation, since the exchange capacity was affected so slightly. It is more likely that groups other than the hydroxyl, e.g., carboxyl groups, play a dominant role in the exchange reaction of the humic acid.

*Titration*s

The titrations, in each instance, were carried out by placing the equivalent of 1 gm. of humic acid, on the oven-dry, ash-free basis, in each of four 250-cc. Erlenmeyer flasks. To each flask was added 75 cc. of CO₂-free water, and flask 1, containing the humic acid and distilled water, was set aside. To flasks 2, 3, and 4, respectively, were added 4, 8, and 12 cc. of 0.1 *N* sodium hydroxide. In addition, each flask contained five drops of toluene. The flasks were then placed in the mechanical shaker for 24 hours. At the end of this period the pH and the resistance were determined by a glass electrode and a salt bridge, respectively. The reaction between the humic acid and alkali is slow, because of the heterogeneity of the colloidal dispersion and, to a less extent, the state of aggregation of the acid. Equilibrium was not established until the suspensions had been subjected to 20 hours of continuous shaking. To each flask was added 1 cc. of 0.1 *N* sodium hydroxide from a burette, the flasks were shaken for 24 hours, and the reaction and resistance were again determined. This procedure was repeated daily until at least 4 cc. of sodium hydroxide had been added to each suspension. Each titration was run in duplicate.

Conductimetric titrations. The salt bridge used in the conductimetric titrations did not possess any great precision, and the conductimetric curves

consequently cannot be relied upon to any great extent. They do indicate very definitely, however, that the sodium hydroxide is reacting with something, undoubtedly hydrogen ions (fig. 1). Curve D, figure 1, represents the dilution curve obtained by adding base in 1-cc. portions to 75 cc. of distilled CO_2 -free water. Various methods were tried in an attempt to make the indicated

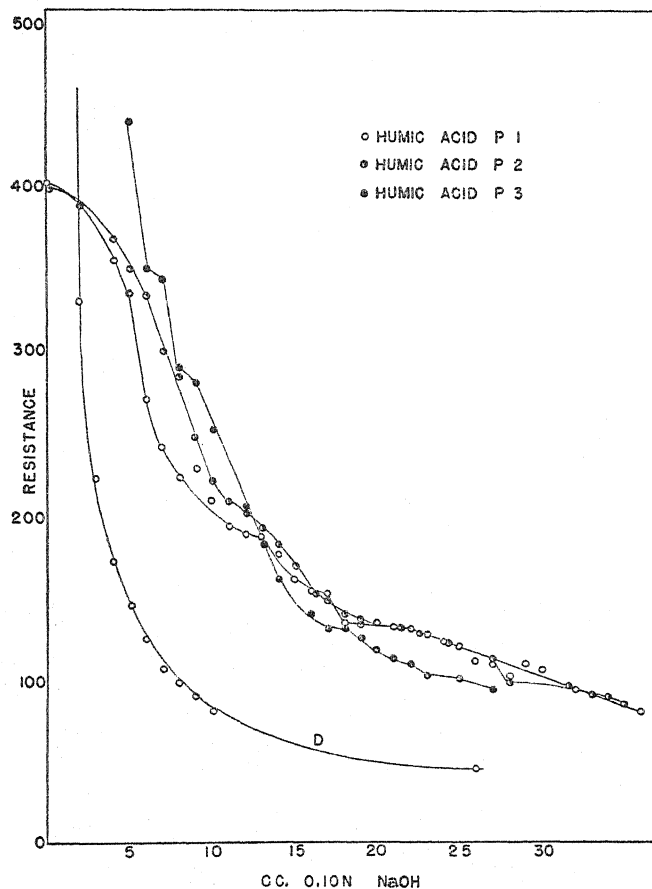


FIG. 1. CONDUCTIMETRIC TITRATION CURVES OF HUMIC ACIDS WITH ALKALI

D, represents the dilution curve. With the exception of figure 6, the equivalent of 1.0 gm. of material on the oven-dry, ash-free basis was used in every titration

breaks more pronounced; e.g., humic acid P2 was leached with sulfuric acid, washed with water, and titrated conductimetrically with barium hydroxide. The resultant curve showed no improvement over those in figure 1.

Data from the conductimetric titrations indicate that humic acids P1 and P2 possess end points at 65 and 70 m.e. of NaOH per 100 gm. of humic acid, respectively. These are indicated by the curves for P1 and P2, in figure 1.

Inflections in these two curves also occur at 175 and 275 m.e. of alkali per 100 gm. of P1 and P2 respectively. Whether these latter points represent end points could not be said without further verification; however, it is interesting to note that corresponding inflections occur in the potentiometric curves P1 and P2, figures 2 and 3.

Potentiometric titrations. All of the potentiometric curves are plotted in figures 2 to 6. The curves for P1, Me1 and Ac1, are plotted in figure 2, and all three show a very definite inflection between pH 4.3 and 5.0. These stoichiometric points lie between 5.5 and 7 cc. of 0.1 *N* base. The curve P1 possesses two distinct breaks, one at pH 4.8, 65 m.e. of NaOH per 100 gm., and

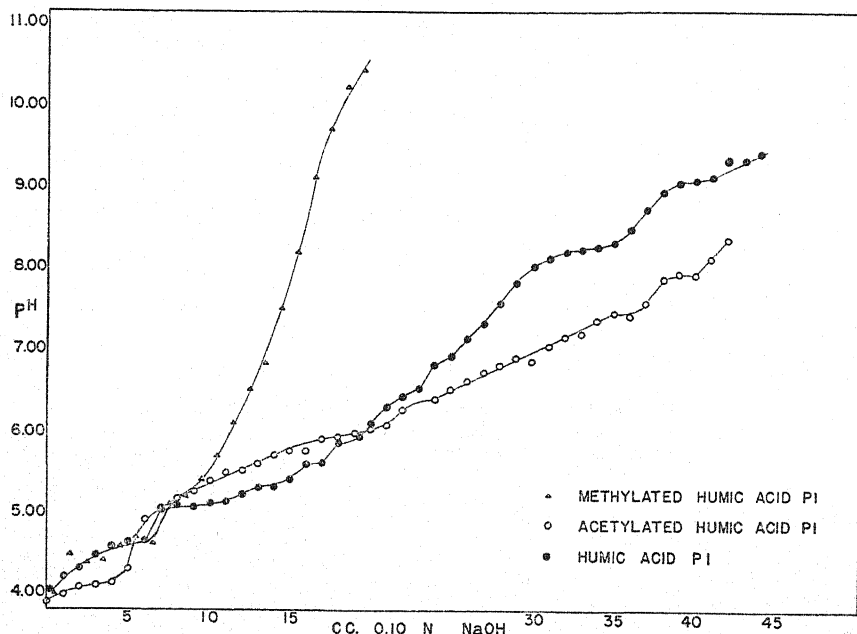


FIG. 2. POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID P1, METHYLATED P1, AND ACETYLATED P1 WITH DILUTE SODIUM HYDROXIDE

the other at pH 8.7, 370 m.e. per 100 gm. Slight inflections in the curve P1 are noted at pH 5.7 and 6.65, 175 m.e. and 235 m.e. of alkali per 100 gm. of humic acid respectively, and although these are not pronounced it should be pointed out that they are five to six times as large as the experimental error involved in determining the pH. The point at pH 5.7, 175 m.e. of NaOH per 100 gm. of humic acid, was indicated in the conductimetric curve for P1 (fig. 1). The very definite inflection occurring at pH 8.7 is reflected in the curve for the acetylated product. Since it occurs in both curves and at different pH levels it indicates that the end point is caused by the humic acid and not by carbonic acid.

Apparently methylation has blocked off all acidic groups with one exception, that being a group not methylated by dimethyl sulfate under the conditions employed. The curve for Me1 (fig. 2) has a sharp inflection at pH 4.9, 70 m.e. of alkali per 100 gm. of material. Above pH 5.1 the methylated humic acid possesses almost no buffering capacity, and the curve ascends sharply.

Acetylation, on the other hand, was not nearly so effective in inhibiting the acidic properties of the acid. The curve for Ac1 (fig. 2) shows a much greater inflection around pH 4.6 than either the Me1 or the P1 curve. The group causing this inflection can therefore not be acetylated by acetic anhydride.

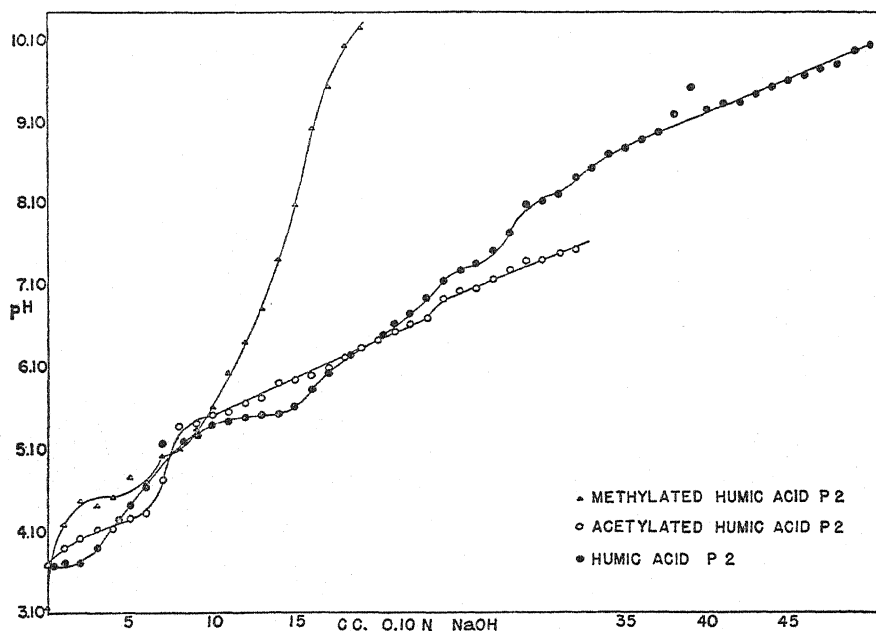


FIG. 3. POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID P2, METHYLATED P2, AND ACETYLATED P2 WITH DILUTE SODIUM HYDROXIDE

The other inflection, at pH 7.65, is also probably due to the presence of some group which was not acetylated by the procedure used. Curiously, acetylation increased the buffering capacity of the humic acid. At pH 6.0 the Ac1 curve crosses the P1 curve and remains below the latter throughout the remainder of the titration.

The potentiometric curves for P2, Me2, and Ac2 are plotted in figure 3. Again we note that all three materials possess a rather definite end point at a relatively low pH, between 3.7 and 5.5 corresponding to 6 and 7 cc. of 0.1 *N* NaOH. Curve P2 possesses two definite inflections, as did curve P1 (fig. 2). The first inflection in curve P2, which occurs at pH 4.7, 62 m.e. of NaOH per 100 gm. of humic acid, is not so abrupt as that for P1, and yet it is more marked

than that in the titration curves for certain dibasic acids, such as *o*-phthalic, succinic, and tartaric. The second point of inflection in curve P2 (fig. 3) occurs at pH 7.7, 280 m.e. of alkali per 100 gm. of humic acid.

Curve Me2 (fig. 3) shows an inflection at pH 4.8, 65 m.e. of alkali per 100 gm. of humic acid, and indicates that humic acid P2, like humic acid P1, possesses a group which is not methylated by methyl sulfate. Again methylation has destroyed the buffering capacity of the acid above pH 5.10.

Acetylation of P2 has made the first inflection much more pronounced, the end point occurring at pH 4.8, while the second inflection noted in the curve for P2 has been eliminated. Apparently humic acid P2 possesses an acidic

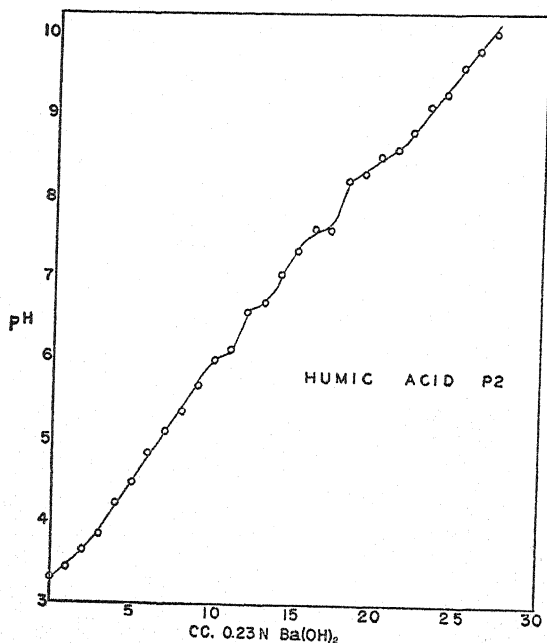


FIG. 4. POTENTIOMETRIC TITRATION CURVE OF HUMIC ACID P2 WITH BARIUM HYDROXIDE

group which is readily acetylated by acetic anhydride. Above pH 6.4 the buffering capacity of P2 has been slightly increased by acetylation.

When P2 was titrated with 0.23 *N* barium hydroxide the curve obtained was very nearly a straight line (fig. 4). The barium ion apparently flocculated the humic acid to such an extent the reaction could not take place. It is interesting to note that the slight inflection indicated in the curve (fig. 4) occurs at 17.5 cc. of barium hydroxide. This is equivalent to 40 cc. 0.1 *N* sodium hydroxide, at which point a slight inflection or disturbance of the curve was indicated in curve P2 (fig. 3).

Figure 5 shows the potentiometric curves for P3, Me3, and Ac3. Apparently this humic acid possesses a stoichiometric point on the acid side of the titration,

similar to that for P1 and P2. The first inflection for P3 is very gradual, and the end point is only an approximation, at pH 4.6. A second and more marked inflection occurs at pH 5.8.

The curve for Me3 (fig. 5) indicates a more abrupt change in the hydrogen-ion concentration than was shown by the curve P2, the end point occurring at pH 4.9. This curve closely resembles those for Me1 and Me2.

With one exception, acetylation of P3, as was the case with P2, effectively blocked the acidic groups, for the curve beyond pH 5.5 is relatively smooth.

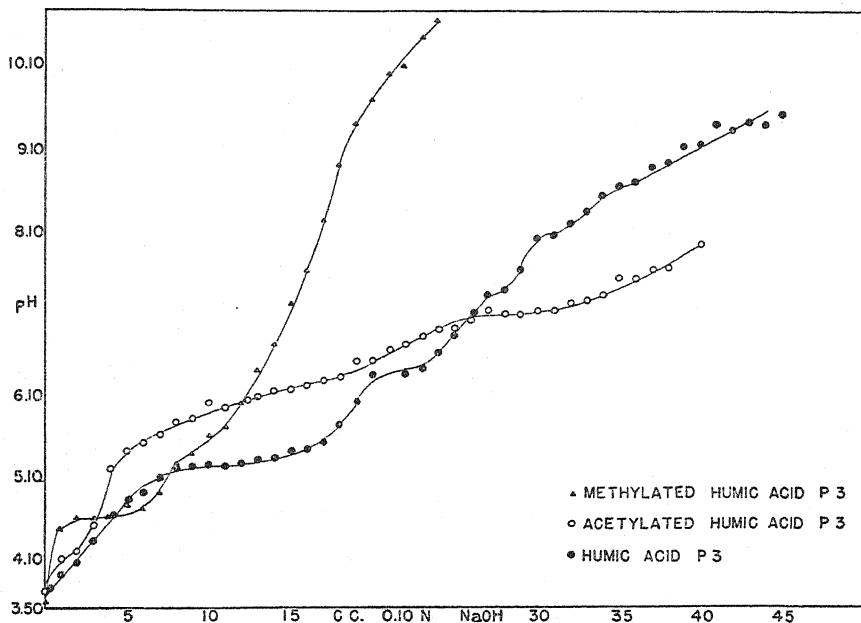


FIG. 5. POTENTIOMETRIC TITRATION CURVES OF HUMIC ACID P3, METHYLATED P3, AND ACETYLATED P3 WITH DILUTE SODIUM HYDROXIDE

The inflection in the Ac3 curve is more abrupt than that in the curve P3, and it indicates that the end point occurs at pH 4.8.

It is held by some investigators that points of inflection in the titration curves of weak acids are apparent only when the acid is added to alkali. In an attempt to check on certain points of inflection in the potentiometric curves, definite volumes, 25 to 35 cc., of 0.1 *N* sodium hydroxide were placed in small Erlenmeyer flasks, to each of which were added minute amounts of the solid humic acid. The suspensions were mechanically shaken for 24 hours after each addition of humic acid, and the pH was then determined. The results are plotted in figure 6. The curves indicate that a reaction is taking place between the humic acid and base and show definite end points in a few instances. The points of inflection for P1 at pH 8.8 and for P3 at pH 5.6 check the breaks obtained in the other curves fairly closely. The breaks indicated

for P1 at pH 7.2 and for P2 at pH 8.1 do not check the stoichiometric points in the other curves quite so well.

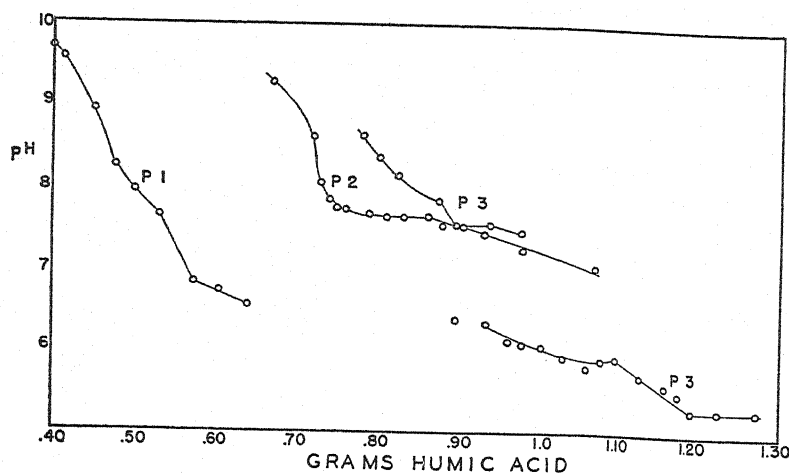


FIG. 6. TITRATION CURVES OF SODIUM HYDROXIDE WITH HUMIC ACIDS P1, P2, P3

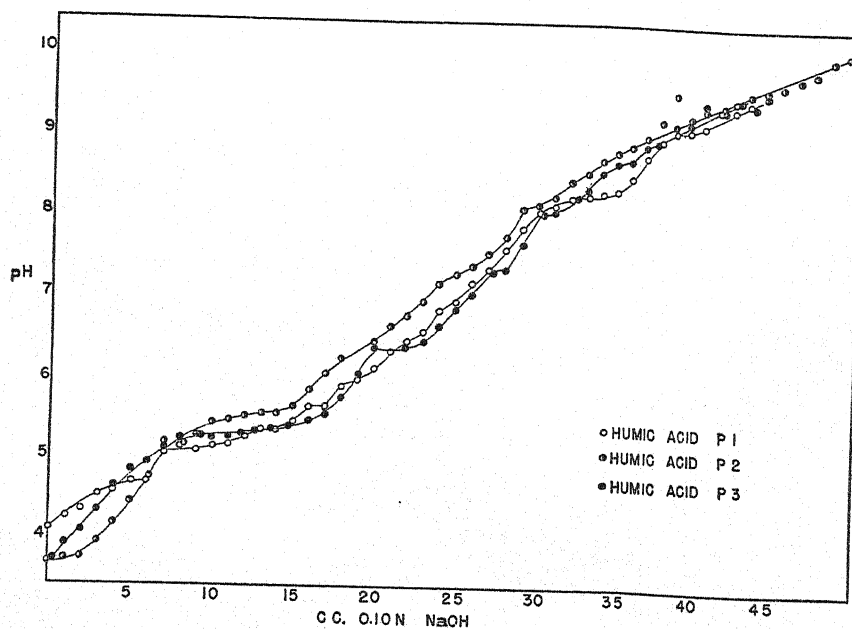


FIG. 7. TITRATION CURVES OF HUMIC ACIDS P1, P2, P3 WITH SODIUM HYDROXIDE SOLUTION

In order to show the great similarities exhibited by the potentiometric curves for the three acids, these curves have been plotted in figure 7. Apparently they differ only in the location of their respective inflection points.

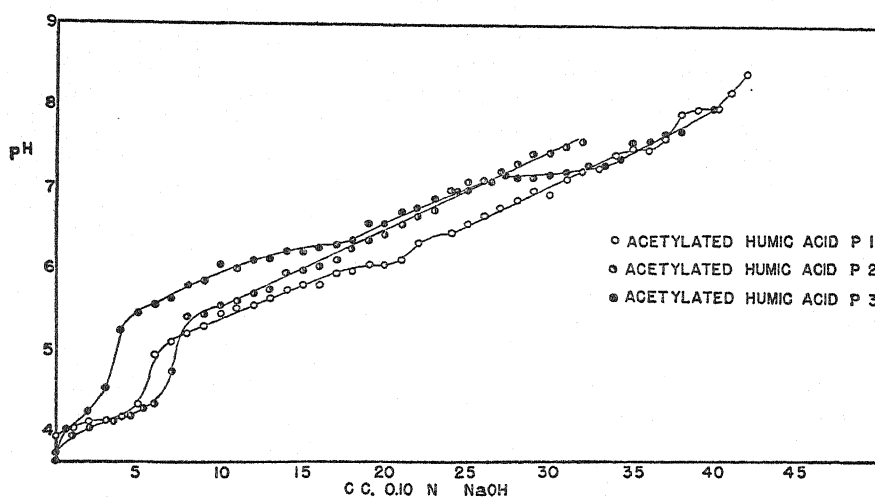


FIG. 8. TITRATION CURVES OF THE ACETYLATED HUMIC ACIDS WITH SODIUM HYDROXIDE SOLUTION

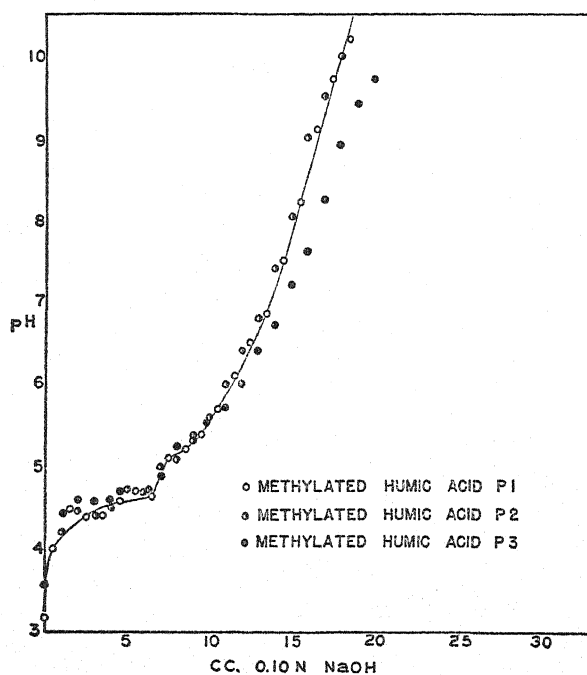


FIG. 9. TITRATION CURVE OF ALL THREE METHYLATED HUMIC ACIDS WITH SODIUM HYDROXIDE SOLUTION

Similarly the potentiometric curves for the three acetylated products have been plotted in figure 8. They show the definite end points for Ac1, Ac2, and Ac3 occurring at pH 4.6, 4.8, and 4.8, respectively. Acetylation did not

diminish the buffering capacity of the humic acids, but it did tend to smooth out the potentiometric curves.

Finally, the potentiometric curves for the three methylated products have been plotted in figure 9. The most striking figure of the group, this shows graphically that the methylated products of all three humic acids are almost identical in their behavior toward titration. Since the acids are probably synthesized from the same central nucleus, and methylation has completely blocked most of the acidic groups, which were formed by partial degradation of the central nucleus, it is reasonable to suspect that the titration curves of the methylated products would be nearly identical.

TABLE 5

Points of abrupt change in the potentiometric titration curves of the humic acids and of the acetylated and methylated humic acids

HUMIC ACID	pH	M.E. OF NaOH PER 100 GM. HUMIC ACID	ACETYLATED PRODUCT	pH	M.E. OF NaOH PER 100 GM. MATERIAL	METHYLATED PRODUCT	pH	M.E. OF NaOH PER 100 GM. MATERIAL
P1	4.8	65	Ac1	4.6	54	Me1	4.9	70
	8.7	65* 370		7.6	370			
P2	4.7	62	Ac2	4.8	70	Me2	4.8	65
	7.7	70* 280 275*						
P3	4.6†	40†	Ac3	4.8	36	Me3	4.9	70
	5.8	185						

* End point indicated by conductimetric measurements.

† Approximate.

The points of abrupt change in the concentration of the hydrogen ions when the humic acids and their acetylated and methylated acids were titrated with 0.1 *N* NaOH are summarized in table 5.

DISCUSSION

Various workers have demonstrated that the nitrogen in the humic acid fraction is present in a proteinlike combination. By use of the conventional factor 6.25 and data from table 1, humic acids P1, P2, and P3 will be found to contain, respectively, 37, 34, and 38 per cent of proteinlike material and 63, 66, and 62 per cent of a complex containing no nitrogen. By use of an average figure for plant protein, the composition of this remaining complex was calculated, and is given in table 6.

These fractions containing no nitrogen are very similar to lignin insofar as

carbon is concerned but are slightly lower in content of hydrogen. Apparently there is a relationship between the humic acid of the soil and lignin, a relationship which is indicated by certain chemical reactions.

Any differences that may exist between the three humic acids are slight, and it was thought probable that these differences might be illustrated by the chemical reactions and analyses used in this investigation. The acetyl content of all three acids was found to be practically identical. Their behavior towards acetylation was very similar to that of lignin isolated from soil and from corncobs. Apparently humic acids P2 and P3, from muck and forest soils respectively, can be acetylated more readily and more completely than humic acid P1, from prairie soil. The acetyl content of P2 and P3 was increased by acetylation 297 and 283 m.e., respectively, per 100 gm. of sample, as compared to 184 m.e. for P1. This variation might be explained by assuming

TABLE 6
Comparison of analyses of several organic complexes

	PER CENT C	PER CENT H	PER CENT N	PER CENT O
P1 minus protein.....	62.0	3.1	0.0	34.9
P2 minus protein.....	62.3	4.8	0.0	32.9
P3 minus protein.....	57.7	4.8	0.0	38.3
Humic acid (peat)*.....	58.2	4.3	1.0	36.5
Humic acid (soil)*.....	56.	5.1	5.4	33.5
Plant protein*.....	52.	7.0	16.	25.0
Cellulose*.....	44.4	6.2	0.0	49.4
Lignin†.....	61-64	5-6	0.0	30‡

* Data from Marshall (5, p. 104).

† Data from Norman (8, pp. 159-166).

‡ Approximate.

that P1 lacks certain groups characteristic of P2 and P3 or that the nature or number of these groups, if present in P1, is very different. This difference is indicated in the potentiometric curves plotted in figure 8. The inflection in the curve Ac1 at a relatively high pH shows that P1 contains some acidic group which was not acetylated by the procedure used.

Lignin isolated from a prairie soil by alcoholic normal sodium hydroxide solution (14) contained the same percentage acetic acid as did the humic acids, but the acetic acid content of corncob lignin was very much lower. The acetic acid content of soil lignin and corncob lignin was increased by acetylation 356 and 223 m.e. respectively, per 100 gm. of sample.

The methoxyl contents of all three humic acids and soil lignin were found to be low, approximately 1.5 per cent, and almost identical, while those for corncob lignin and oat hull lignin were respectively, 12.9 and 15.6 per cent. Apparently, in the formation of the humic acids and soil lignin from plant lignin, considerable loss of methoxyl groups took place. The methoxyl content

of soil lignin, corncob lignin, and oat hull lignin can be increased by methylation by approximately 520 m.e. per 100 gm. of sample, whereas the increase in methoxyl content for each humic acid is approximately 228 m.e. per 100 gm. of acid.

It has been shown that methylation and acetylation decreased the base-exchange capacity of all three acids. The decrease, however, is much less than equivalent to the increase in acetyl and methoxyl content. This clearly indicates the presence of hydroxyl or other groups which readily undergo methylation and acetylation but which are not involved in the base-exchange reaction. Corncob lignin and soil lignin likewise possess many groups which are readily acetylated and methylated but which are not active in the base-exchange reaction. Since the base-exchange capacity of both lignins, however, was reduced tremendously by acetylation and methylation, it is apparent that these reactions are centered, to a large degree, in the same groups that are responsible for the exchange reaction. This is in contrast to the behavior of the humic acids.

It would appear, then, that the base-exchange reaction of lignin deals primarily with enolic and weakly acidic phenolic hydroxyl groups, while the reactions of methylation and acetylation deal with alcoholic as well as enolic and weakly acidic hydroxyl groups. In contrast, the exchange reaction of the humic acid is centered in the carboxyl and acidic phenolic groups, whereas methylation or acetylation, in general, involves a reaction with the alcoholic hydroxyl groups.

Plant lignin when incorporated in the soil decomposes very slowly. As it is slowly modified, it combines with a nitrogenous material to form humic acid. The partial degradation of lignin consists of demethylation, probably hydrolysis of certain groups, fission of some specific rings or linkages, and various other chemical reactions that would result in the formation of acidic groups, such as carboxyl or phenolic hydroxyl groups. These, being active in the base-exchange reaction, would greatly increase the exchange capacity of the resultant complex, humic acid. It would, however, still possess groups which could be methylated and acetylated but which would be inactive toward base exchange. Relative to plant lignin the capacity for methylation of humic acid is very much smaller. Therefore, during the formation of humic acid from lignin there has been a loss or rearrangement of certain groups, or a linking of the nonnitrogenous portion to the proteinlike complex through these groups, which has reduced the capacity of the humic acid to be methylated.

The potentiometric curves illustrate the striking similarity, and a few minor differences, between the three humic acids. All three titration curves for the acids possess a point of inflection around pH 4.7. Methylation and acetylation failed to eliminate the break in the curve, which no doubt is due to the presence of a carboxyl group in the humic acid. The results do not exclude the possibility that we may have titrated a mixture of acidic materials.

With one exception, acetylation tended to smooth the titration curves of the humic acids beyond pH 4.7. Evidently certain weakly acidic groups were

blocked by esterification. This might explain the fact that the potentiometric curves for the acetylated products possess the most pronounced inflection points, around pH 4.7, of all the titration curves. The break in the curve for each humic acid might be influenced by several acidic groups, e.g., enolic or phenolic hydroxyl groups. Acetylation of these would leave the carboxyl group free to react with the alkali, and the curve should show a more distinct point of inflection. Virtually the only distinction noted between the three humic acids was indicated in the potentiometric curves for the acetylated products and in the base-exchange capacity. As has been pointed out, P1 possesses some acidic group not common to humic acids P2 and P3. This group is readily methylated but is not acetylated by acetic anhydride. The base-exchange capacity of P1 is greater than that of either P2 or P3, a fact which might be attributed to the presence of this acidic group.

The neutralization capacity, or the adsorption capacity of these acids at neutrality, can readily be calculated from the curves. The titration curves intersect a line drawn horizontally from pH 7.0 at a definite point, corresponding to a certain volume of 0.1 *N* sodium hydroxide or to a given number of milliequivalents of sodium hydroxide per gram of acid. The amount of adsorbed base on a milliequivalent basis was found to agree in a general way with the exchange capacity for the humic acids and acetylated products. These values are tabulated below for the sake of comparison.

SAMPLE	NEUTRALIZATION CAPACITY	BASE-EXCHANGE CAPACITY
	M.e. of NaOH per 100 gm.	Average m.e. of Ca and Ba per 100 gm.
P1	270	394
P2	249	274
P3	271	253
Ac1	305	342
Ac2	252	248
Ac3	250	224
Me1	135	273
Me2	134	227
Me3	148	246

There is a wide discrepancy between these two values for the methylated products. Methylation was so effective in destroying the buffering capacity of the acids, and the potentiometric curves were so similar, that the neutralization capacity of each methylated product was decreased by about 100 m.e. per 100 gm. of sample.

SUMMARY

Humic acid was extracted and studied rather extensively from three soil groups; namely, gray-brown forest soils, muck soil, and grassland soils of

the Prairie, Chestnut, and Chernozem regions. The three humic acids were found to be very similar in all physical and chemical properties.

The humic acids isolated were negatively charged hydrophilic colloids. The conductivity of these, suspended in distilled water, was low, ranging from 400 to 2000 reciprocal ohms.

Combustion analysis showed their elementary compositions to be very similar.

The acetyl and methoxyl contents of the humic acids were low and were almost identical with those of lignin extracted from a prairie soil by means of alcoholic sodium hydroxide.

The humic acids were readily acetylated and methylated. Plant and soil lignins were methylated much more completely than the humic acids, but acetylation of the acids took place as readily and completely as with the plant lignin.

The base-exchange capacity for all three humic acids was high. The exchange capacity was reduced slightly by acetylation and methylation, but the reduction was much less than equivalent to the increase in acetyl and methoxyl content.

All three humic acids possess hydroxyl groups which can be esterified or methylated but which do not undergo base-exchange reaction. These may be alcoholic, enolic, or weak phenolic groups.

The potentiometric titration curves indicate the presence of a carboxyl group in each humic acid. Potentiometric titrations of the humic acids with barium hydroxide were not successful, since the barium ion flocculated the acids to such an extent the reaction could not proceed. The differences noted between the humic acids were very few and dealt with slight variations in base-exchange capacities and titration curves. Humic acid P1, from prairie soils, contained an acidic group which was not found in the other two and which could not be esterified by acetic anhydride. It also had the highest base-exchange capacity of the three acids. Humic acids P2 and P3, from muck and gray-brown forest soils respectively, were more readily and more completely acetylated than was humic acid P1.

The slight differences noted probably are due to the presence or absence of certain minor groups in the complex. When these differences were minimized by methylation the humic acids were proved to be almost identical.

The study indicated that the nonnitrogenous fraction of the humic acid consists of a slightly modified lignin complex. This modification is probably brought about primarily by demethylation, hydrolysis, and other chemical reactions which would cause the formation of phenolic and carboxyl groups.

REFERENCES

- (1) GILLAM, W. S. 1939 The geographical distribution of soil black pigment. *Jour. Amer. Soc. Agron.* 31: 371-387.
- (2) GORTNER, R. A. 1916 The organic matter of the soil: II. A study of carbon and nitrogen in seventeen successive extracts; with some observations on the black pigment of the soil. *Soil Sci.* 2: 539-548.

- (3) McGEORGE, W. T. 1930 The base exchange property of organic matter in soils. *Ariz. Agr. Exp. Sta. Tech. Bul.* 30.
- (4) McGEORGE, W. T. 1931 Organic compounds associated with base exchange reactions in soils. *Ariz. Agr. Exp. Sta. Tech. Bul.* 31.
- (5) MARSHALL, C. E. 1935 *Colloids in Agriculture*. Edward Arnold and Co., London.
- (6) MILLAR, H. C., SMITH, F. B., AND BROWN, P. E. 1936 The base exchange capacity of decomposing organic matter. *Jour. Amer. Soc. Agron.* 28: 753-766.
- (7) MULLER, J. F. 1933 Some observations on base exchange in organic materials. *Soil Sci.* 35: 229-237.
- (8) NORMAN, A. G. 1937 *Biochemistry of Cellulose, Polyuronides, Lignin, etc.* Clarendon Press, Oxford.
- (9) PERKINS, A. G. 1905 The determination of acetyl groups. *Jour. Chem. Soc.* 87: 107-110.
- (10) PHILLIPS, M. 1927 The chemistry of lignin: I. Lignin from corncobs. *Jour. Amer. Chem. Soc.* 49: 2037-2040.
- (11) PHILLIPS, M. 1932 The chemistry of lignin: IV. Lignin from oat hulls. *Jour. Amer. Chem. Soc.* 52: 793-797.
- (12) PHILLIPS, M. 1932 The quantitative determination of methoxyl, lignin and cellulose in plant materials. *Jour. Assoc. Off. Agr. Chem.*, 15: 118-131.
- (13) WAKSMAN, S. A. 1936 Origin and nature of soil organic matter. *Soil Sci.* 22: 421-436.
- (14) WELDON, M. D. 1937 The Extraction and the Chemical Nature of Lignin from a Prairie Soil. Lincoln, Nebraska. (Doctoral Diss.)

THE pH OF SOIL AS AFFECTED BY SOIL MOISTURE AND OTHER FACTORS^{1, 2}

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At the Citrus Experiment Station, Riverside, there is a 9-year-old navel orange orchard on which differential irrigation experiments have been conducted since 1933. Studies in the control of soil moisture necessitate the taking of a large number of soil samples at regular intervals. Haas (7) determined the pH range for the healthy growth of citrus in sand, soil, and solution cultures and was desirous of studying the pH range for healthy growth in the field. By utilizing samples taken in the irrigation program for the determination of pH, a considerable saving was accomplished.

The soil in the experimental orchard is classified as Ramona sandy loam and has a moderately compact stratum at a depth of from 2 to 3 feet. Prior to the planting of the orchard in June, 1930, the field had been utilized in the growing of grain by dry farming. Beginning with the second year at the orchard, 5 pounds of ammonium sulfate per tree per annum was applied.³ Organic matter has been applied three times, twice as barnyard manure (0.5 pound of nitrogen per tree) and once as bean straw (0.5 pound of nitrogen per tree). A mixed cover crop has been grown each winter and turned under in spring.

Most of the orchard is furrow irrigated, and a small part is basin irrigated. Water of moderately low salt content and with a measured pH range of 7.8 to 8.5 has been used at all times for irrigation. Algae had an influence on the pH of the water.

At first, pH determinations were made on suspensions (1-5 soil-water ratio) of samples that had been used in determining soil moisture in the differentially furrow-irrigated plots. Even though it soon became apparent that the pH values at such dilutions were not representative of the values prevailing under the moisture conditions in the orchard, the determinations were continued on that basis. Subsequently pH determinations were made *in situ* and on drawn samples in both the furrow- and basin-irrigated plots. The pH deter-

¹ Paper No. 408, University of California Citrus Experiment Station, Riverside, California.

² It is recognized that Sorensen's definition of pH applies to an aqueous solution and not necessarily to a two- or a three-phase system. The values recorded are the readings obtained by using pH meters.

³ Beginning in 1938, some changes in fertilizer practice were made on certain basin-irrigated plots, as indicated in subsequent sections of this paper.

minations on drawn samples at field moisture content were made with the spear-type glass electrode as developed by McGeorge (17), and as used with a Coleman pH meter (type 3D); determinations of pH *in situ* were made with a Beckman (extension type) shielded glass electrode and a companion calomel electrode. These electrodes permitted determinations at a distance of 10 feet from the pH meter. For measurements made with the Coleman instrument, the temperature of the samples and of the machine was that of the room; for the determinations of pH of soil *in situ*, the machine was adjusted to the temperature of the soil. Distilled water was used at all times during these studies, and the instruments were checked at frequent intervals against suitable buffer solutions of known pH values.

Absence of a standardized technic in determining the pH of soils necessitated a study of some of the factors affecting the pH of the sample.

EFFECT OF DRYING UPON pH OF SOILS

An extensive study of the effect of air drying on the pH of soil was carried on by Bailey (2), who employed the bubbling hydrogen electrode to determine the pH of 327 soil samples. These samples were collected throughout the United States and Canada and were transported in tin containers with tight-fitting covers. In the procedure used by Bailey the soil samples in every case were thoroughly mixed. One part (by volume) of soil to two parts of boiled distilled water was used with the medium- and light-textured soils, and four parts of boiled distilled water with clays. The method employed in the determination of pH largely eliminated the influence of CO₂. The determinations, made on both the fresh and the air-dried samples, led Bailey to the following conclusions: The pH of the soil suspension was not affected by the length of time the water was in contact with the soil sample, a conclusion substantially in agreement with the results of Dean and Walker (6); air drying generally lowered the pH;⁴ and soil samples should be air-dried prior to dilution in the determination of pH.

The volume method gives no consideration to the residual moisture in the soil sample. As shown by Salter and Morgan (23), McGeorge (18), Keaton (12), and in this paper, the degree of dilution is an important factor in regard to the pH value. From a study of the pH of displaced soil solutions, Wilcox (25) has suggested that in the use of extractions for pH determination, the lowest ratio of water to soil be used.

As previously mentioned, the routine soil samples collected and oven dried for moisture determinations were available to us for study. To learn whether oven-dried samples could be used satisfactorily, a comparison was made of the pH of oven-dried and air-dried samples. Table 1 gives the results (on a 1-5 soil-water ratio basis) for samples taken in a furrow-irrigated plot of the ex-

⁴ McGeorge (16) found that the pH values of alkaline calcareous soils were increased by air drying as compared with determinations made on fresh soil.

perimental orchard. Samples in close proximity to each other were taken from ten holes, each to a depth of 4 feet. Each soil sample was thoroughly mixed and divided; one portion was air dried while another was oven dried. The pH values of the air-dried soil samples were consistently higher than those of the oven-dried samples. Although McGeorge employed a temperature of 350°C. for drying samples of alkaline calcareous soils as compared with 100°C. used by Joseph and Martin (11) in drying alkaline Egyptian soils, or with 105°C. in the present experiments, the results all agree in that lower pH values were obtained with heated soils than with air-dry soils. The variability of the values obtained in the present experiments on the oven-dry samples was no greater than that for the air-dry samples. It was feasible, therefore, to

TABLE 1
pH Values of oven-dried and air-dried Ramona sandy loam
(1-5 Soil-water ratio)

HOLE NUMBER	DEPTH, 1 FOOT		DEPTH, 2 FEET		DEPTH, 3 FEET		DEPTH, 4 FEET	
	Oven dry	Air dry	Oven dry	Air dry	Oven dry	Air dry	Oven dry	Air dry
1	6.74	6.81	7.17	7.34	7.35	7.38	7.26	7.67
2	6.03	6.33	7.08	7.44	7.19	7.46	6.98	7.37
3	6.56	6.63	7.14	7.36	7.34	7.70	7.00	7.53
4	6.68	6.82	6.77	7.27	7.23	7.63	7.36	7.65
5	7.35	7.87	7.35	7.83	7.17	7.60	6.88	7.13
6	7.19	7.54	7.47	7.89	7.48	7.97	7.54	7.89
7	6.73	6.80	7.24	7.56	7.57	8.01	7.63	8.07
8	6.08	6.13	7.00	7.11	7.10	7.23	7.30	7.33
9	7.07	6.98	7.22	7.18	6.93	7.01	7.42	7.50
10	6.59	6.62	7.24	7.58	7.27	7.60	7.20	7.51
Av.....	6.52	6.64	7.13	7.39	7.24	7.46	7.20	7.49

use the oven-dried samples to determine the relative changes in the pH of soils under various irrigation treatments.

To determine the effect of duration of oven-drying on the pH of soil, four different soils in the air-dry condition were used. Each soil was thoroughly mixed and screened, and certain fractions of the soils were then oven-dried (105°C.) for 17½ and 118 hours, respectively. Determinations of pH were made at the 1-5 and 1-10 soil-water ratios. Table 2 presents the results obtained. Prolonged heating produced lower pH values in both the 1-5 and 1-10 dilutions of Aiken clay loam, a primary soil derived from basic igneous rocks, and of Ramona sandy loam. Unlike the Aiken and Ramona soils, Tujunga sand, a raw soil derived from granitic rock high in quartz, and Traver clay, the sample of which was highly saline, showed no consistent changes in pH values after prolonged heating.

EFFECT OF STORAGE ON pH

Since the data in table 1 show that the pH values of the air-dried samples were higher than those of oven-dried samples, and since it is not always practicable to make the pH determinations promptly after soil sampling, it is desirable to know what, if any, changes in pH occur during storage. Samples from the navel orange orchard were obtained from ten holes to a depth of 4 feet, five holes on April 15 and five on April 23, 1938. Half of the samples (table 3) were initially oven-dried, and pH determinations were made on a part of each sample; the remainder of the sample in each case was air-dried for a period during which pH determinations were made on portions of the sample; and finally pH determinations were made on the oven-dried remainder. The other half of the samples (table 3) were initially air-dried, and

TABLE 2
Effect of duration of oven drying (105°C.) on pH of various soils

SOIL	SCREEN SIZE, NUMBER*	SAMPLE SIZE	DILUTION (SOIL-WATER RATIO)	DRIED 17½ HOURS		DRIED 118 HOURS	
				Sample A	Sample B	Sample A	Sample B
Aiken clay loam†	20-40	30	gm.				
			1-5	6.01	6.09	5.86	5.85
			1-10	6.17	6.15	5.93	5.90
Tujunga sand	40-60	30	1-5	6.45	6.40	6.60	6.60
			1-10	6.50	6.53	6.43	6.42
Ramona sandy loam	40-60	30	1-5	7.52	7.52	7.32	7.31
			1-10	7.47	7.50	7.29	7.22
Traver clay	60-80	30	1-5	10.61	10.61	10.61	10.61
			1-10	10.60	10.60	10.57	10.57

* 20-40 indicates material passed a 20-mesh and was caught on a 40-mesh screen.

† Soil high in organic matter.

pH determinations were made over a period of time on portions of the sample; and finally the remainder of each sample was oven-dried, and pH determinations were again made. In table 3 the soils in the two series represent different samples, whereas those in table 1 are portions of uniformly mixed samples. The average initial pH values (table 3) of the air-dried and oven-dried samples taken April 15 and April 23, 1938, were approximately the same.

In the first series, oven-dried for 48 hours, subsequent storage in paper bags brought about an increase in pH. When these samples were again oven-dried, the pH values were approximately those of the original oven-dried soil. In the second series, continued storage in the air-dry condition was accompanied by higher pH values than those of the initial air-dried soil. Oven drying of the samples which had been stored for nearly a year caused the pH to be

lowered to nearly the original value. The pH of air-dry samples fluctuated considerably during the storage period.

TABLE 3

Changes in pH produced in stored samples of Ramona sandy loam obtained from an orchard (1-5 Soil-water ratio)

HOLE NUMBER	DEPTH	TREATMENT OF SAMPLES COLLECTED 4/15/38 AND OVEN DRIED				TREATMENT OF SAMPLES COLLECTED 4/23/38 AND AIR DRIED			
		Oven dried 4/15-4/17 1938	Air dried 4/17-8/4 1938	Air dried 4/17/38- 2/18/39	Oven dried 2/21-2/23 1939	Air dried 4/23-4/30 1938	Air dried 4/23-8/6 1938	Air dried 4/23/38- 2/18/39	Oven dried 2/21-2/23 1939
1	feet								
	1	6.32	6.59	6.92	6.63	6.43	7.08	6.93	6.68
	2	7.13	7.50	7.37	7.12	6.66	7.40	7.19	6.88
	3	6.87	7.32	7.03	6.74	6.77	7.60	7.34	7.12
2	4	6.80	7.32	7.13	6.93	6.86	7.80	7.47	7.15
	1	6.42	6.85	7.00	6.80	6.55	7.42	7.18	6.88
	2	7.16	7.40	7.28	7.15	7.50	7.81	7.50	6.93
	3	7.01	7.39	7.33	7.14	7.78	8.10	7.84	7.48
3	4	7.42	7.70	7.57	7.21	7.35	8.00	7.93	7.34
	1	6.93	7.38	7.25	7.00	7.47	8.00	7.96	7.52
	2	7.55	7.75	7.50	7.32	7.37	8.27	7.97	7.60
	3	7.69	7.88	7.71	7.41	7.49	8.06	7.72	7.34
4	4	7.28	7.53	7.00	6.85	7.48	7.21	7.00
	1	6.47	6.70	6.90	6.70	6.48	7.10	6.92	6.58
	2	7.65	7.83	7.72	7.28	7.32	7.71	7.69	7.37
	3	7.72	8.02	8.10	7.73	7.60	8.09	7.90	7.65
5	4	7.57	7.91	7.73	7.47	7.47	7.89	7.72	7.50
	1	6.68	6.96	6.94	6.63	6.82	7.60	7.41	7.10
	2	7.12	7.55	7.46	7.19	7.38	8.09	7.93	7.50
	3	7.78	7.98	7.87	7.47	7.82	8.24	8.16	7.47
Av. 1-5	4	7.32	7.98	7.78	7.45	7.32	8.10	7.85	7.47
	1	6.52	6.83	6.98	6.73	6.63	7.34	7.15	6.85
	2	7.27	7.58	7.44	7.20	7.12	7.76	7.56	7.16
	3	7.24	7.61	7.44	7.16	7.29	7.96	7.71	7.37
Av.....	4	7.19	7.62	7.47	7.15	7.09	7.79	7.54	7.25
		6.92	7.25	7.28	7.00	6.95	7.64	7.44	7.11

EFFECT OF SIZE OF SAMPLE ON pH

Buehrer and Williams (4), using highly calcareous soils, found a gradual rise in pH on increasing the absolute weights of the soil at the 1-10 dilution. In order to determine whether the pH values of the soils we were using were affected by variations in the absolute weights of samples, experiments were

conducted in which the ratio of soil to water was held constant, while the oven-dry soil weights varied. The same four soil types shown in table 2 were used in these trials. As shown in table 4, the range in size of the samples of screened soil was from 4 to 50 gm. In the 1-5 dilution for the soils tested there were no apparent changes in the pH values regardless of the soil sample size. Determinations of pH values on a 1-10 basis were made also, and comparable

TABLE 4

Effect of size of sample on pH of various soils at 1-5 soil-water ratio, titration-type glass electrode

SAMPLE SIZE	SOIL SERIES			
	Aiken	Tujunga	Ramona	Traver
gm.				
4	6.89
	7.00
5	6.40	6.87	7.78	10.27
	6.40	6.88	7.85	10.25
6	6.43	6.77	7.87	10.27
	6.40	6.86	7.81	10.27
8	6.40	6.90	7.84	10.25
	6.41	6.98	7.87	10.25
10	6.43	6.90	7.90	10.27
	6.45	6.91	7.90	10.27
15	6.47	6.75	7.78	10.30
	6.43	6.79	7.76	10.29
20	6.43	6.82	7.83	10.27
	6.45	6.83	7.85	10.27
25	6.40	6.82	7.80	10.28
	6.40	6.79	7.80	10.29
30	6.44	6.78	7.85	10.31
	6.43	6.80	7.83	10.31
35	6.64
	6.68
40	6.40	6.69	7.83
	6.40	6.80	7.80	10.32
50	6.43	6.78	7.77
	6.40	6.89	7.72	10.32
Av.....	6.42	6.81	7.82	10.28

results were obtained. Unless otherwise specified, 30-gm. samples of soil were used in the laboratory.

EFFECT OF SOIL DILUTION ON CHANGES IN pH

Titration-type glass electrode

Fractions of the same soil types reported in table 4 were used in a study of the effect of dilutions on pH values determined with a titration-type glass electrode. As seen in table 5, the data for the Tujunga soil at any given dilu-

tion within the soil-water range (1-2 to 1-10) show a decrease in pH with increasing dilution. This is what would be expected were the buffer effect overcome by the large amounts of distilled water (pH 5.30) used. For any given size of sample tested, in the other soils studied, there was a small increase in pH as the dilution of the sample was increased. This fact is suggested in the results of Kelley and Brown (14), who found low pH values for extracts of two soils when the ratio of soil to water was 1 to 2, and higher pH values with greater dilution, reaching a maximum with one soil when the ratio was 1 to 10 and with the other soil when the ratio was 1 to 40.

Spear-type glass electrode

The spear-type glass electrode, which is well adapted for measurements of pH values of soils at low moisture content, was used in determining the effect of dilution on samples of soil of several types. The results are shown in table 6.

TABLE 5

*Effect of dilution on pH of oven-dried soils as determined with a titration-type glass electrode**

SOIL†	SOIL-WATER RATIO						pH OF DISTILLED WATER
	1-2	1-3	1-4	1-5	1-8	1-10	
1	6.96	6.87	6.78	6.70	6.53	6.44	5.30
2	6.22	6.40	6.42	6.42	6.51	5.31
3	7.88	8.03	8.19	8.23	8.32	5.31
4	10.03	10.16	10.24	10.27	10.31	5.30

* pH readings made on at least six samples at each dilution for each soil.

† 1, Tujunga (40-60); 2, Aiken (20-40); 3, Ramona (80-100); 4, Traver (80-100): (40-60) indicates soil passed a 40-mesh and was caught on 60-mesh screen.

With the exception of Oakley sand (soil 5) at high dilutions, where there is a reduction in the pH values, there was an increase in pH with dilutions.

Soluble salts, as well as colloids, affect hydrolysis in calcareous soils, according to Buehrer and Williams (4). In their opinion, the calcium carbonate-bicarbonate buffer system determines the pH of calcareous soils.

McGeorge (19), in pointing out the effect of dilution in increasing the pH of soils, has shown that above the 1-10 soil-water ratio there is but little change with dilution, this ratio thus representing the maximum potential pH of the soil. The results reported by McGeorge are applicable to the data (with the exception of soil 5) listed in table 6 and plotted in part in figure 1.

Several of the soils taken from areas in which citrus is grown, though slightly basic at high dilutions, were acid at field moisture content. The maximum pH values of these soils are seldom reached under field conditions, and for learning the approximate pH values at which trees grow in the field it is best to deal with soil-water ratios approximating those found in the field. As a result of studies with solution and soil cultures with citrus, walnut, and avocado

trees under controlled conditions, Haas (8) has concluded that healthy growth in these trees is made in acid rather than basic solutions and that the degree of acidity favorable for the growth of these trees is far greater than is usually assumed. As a result of the practice of determining pH values of soils by the 1-5 soil-water ratio method, it is commonly believed that most soils on which citrus trees are planted in southern California are rather basic (pH 8.0-8.5 or higher). Figure 1 gives some indication that the soils in which healthy citrus

TABLE 6

Effect of dilution on pH of soils as determined with a penetration-type glass electrode

(Range in pH of distilled water 5.09-5.24; moisture percentage expressed on oven-dry basis)

MOISTURE PERCENTAGE	SOIL*								
	1	2	3	4	5	6	7	8	9
5	6.44	7.25	6.48	7.58
8	7.03	5.67
10	6.65	6.49	7.99
15	7.61	6.74	6.91	6.12	6.98	8.09
20	5.56	8.14	8.05	6.46	7.29	8.33
25	6.56
30	5.52	8.31	7.77	6.93	7.03	7.34	8.65
35	6.88
40	7.39	8.88
45	7.00
50	5.85	8.35	7.92	6.99	7.81	7.08	7.53	8.97
55	7.10
60	7.49
65	7.15
70	7.57
80
85	7.62
100	5.85	8.50	8.06	7.14	7.80	7.20	7.34	7.80	9.22
250	6.00	8.56	8.32	7.19	7.29	7.88	8.34	9.37
500	6.11	8.61	8.47	7.24	7.39	7.30	7.97	8.42	9.49
1000	7.83	8.62	9.65

* 1, Aiken clay loam; 2, a saline soil; 3, Yolo sandy loam; 4, Hanford sandy loam; 5, Oakley sand; 6, Ramona sandy loam; 7, Yolo silty clay loam; 8, Altamont clay loam; 9, an alkaline soil from Santa Ana River flood plain. Samples of soil 1 had been oven dried; all other samples air dried.

trees are growing in southern California may be acid rather than basic in reaction.

Determinations of pH values, made on suspensions of soil at high dilutions, allow a degree of hydrolysis rarely, if ever, encountered under field conditions. McGeorge (18), in pointing out that far less acid is required to reduce the pH of a basic soil to 7 at field-moisture content than at 1-10 dilution, has suggested that the reduction of pH in basic soils is a much simpler operation than was indicated previously.

The influence of salts and soil-water ratio was investigated by Puri and Asghar (22), who found that in the absence of salts the pH value of soils is not affected by the soil-water ratio. The pH value of soil is considered to be the result of ionization at the surface of the colloidal particles and hydrolysis of the exchangeable base. These authors recommend that the determination of soil reaction be carried on with *N* KCl solution, whereas McGeorge (16) recommends in one procedure with 1-10 soil-water suspensions the addition of 10 cc. each of 2 *M* NaCl and 2 *M* CaCl₂.

pH of water-displaced solutions of certain soils

In the course of experiments with samples from different soil types, it was desirable to know the composition of the (1-5 soil-water ratio) extracts. Table 7 gives the results of duplicate determinations and shows the wide range of

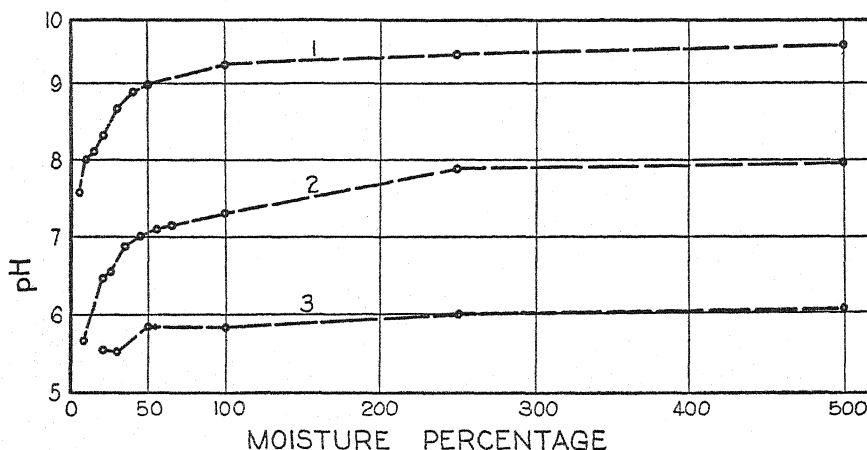


FIG. 1. RELATION OF pH TO SOIL MOISTURE

1. Alkaline soil from Santa Ana River flood plain; 2. Yolo silty clay loam; 3. Aiken clay loam

total solids and the concentrations of the various ions. Although the pH values of many samples of soil in southern California, when determined at the 1-5 soil-water ratio, have been found to give basic reactions to varying degrees, it is of interest to report a pH of 3.25 for a sample of soil at this same soil-water ratio.

Several of the soils reported in table 7 were used in determining the pH of the displaced solutions (table 8). A definite amount of distilled water was thoroughly mixed with weighed samples of air-dry soil. The mixture was then firmly compacted with a flat rubber-stoppered plunger into a glass percolator or soil filter tube from which the clay filter was omitted. A definite volume of distilled water was then added to the top of the soil. The glass percolators operated by gravity, while the filter tubes were used with air pressures in order to obtain the displaced solution (5).

Table 8 gives the pH values of successive aliquots of displaced solution. The variations in pH of these aliquots obtained from a given soil sample were relatively small and generally were of no consequence, even though in some samples a slight trend was sometimes discernible. Table 8 makes it possible to compare the pH values of a given soil sample at approximately the moisture equivalent and at the 1-5 soil-water ratio, with those of successive aliquots of displaced solution.

The pH values of the soil samples were generally lower at the moisture equivalent than at the 1-5 soil-water ratio. The pH of the displaced solution of Yolo sandy loam approximated the pH found at the moisture equivalent, whereas the pH values of the displaced solutions of Ramona sandy loam and Hanford sandy loam were closer to those at the 1-5 soil-water ratio. The pH of the solution displaced by air pressure from Traver loam was higher at the

TABLE 7
Composition of (1-5 soil-water ratio) extracts of certain air-dried soils
(Data reported as p.p.m. in air-dry soil)

SOIL	MOIS- TURE, (DRY- SOIL BASIS)	TOTAL SOLIDS 103°- 105°C.	TOTAL SOLIDS AFTER IGNI- TION	Ca	Mg	Na	K	Cl	SO ₄
	<i>per cent</i>								
Ramona sandy loam..	0.8	368	113	29.8	13.2	268	90.0	73	15.5
Yolo silty clay loam...	3.5	330	150	22.8	6.3	274	63.0	28	Trace
Hanford sandy loam..	0.6	148	40	28.3	5.7	248	50.0	28	Trace
Altamont clay loam..	6.6	585	358	109.5	8.5	227	43.3	26	Trace
Yolo sandy loam....	1.1	1,308	538	161.0	28.5	276	65.5	37	38.0
Alkaline soil from Santa Ana River flood plain.....	0.8	11,958	11,108	71.8	69.4	3,857	652.0	2,770	3,627.0
Traver loam.....	1.2	37,520	36,390	1,889.0	73.2	8,097	2,229.0	718	18,910.0
Woodrow clay.....	...	58,518	58,020	721.0	70.5	19,979	1,391.0	24,881	9,802.0

higher soil moisture content. Other initial soil moisture contents, in the samples used, may greatly alter the pH values obtained. By the oil-pressure method of obtaining the film water from soils of approximately optimum moisture contents, Plummer (21) found the pH values of displaced solutions to be lower in acid soils and higher in alkaline soils than the pH values of dilute soil suspensions. Pierre (20) obtained the same pH for the soil suspension, soil extract, and soil solution. McGeorge (18) has reported that the slower the irrigation water penetrates the soil the higher the pH becomes. The time required for displacement to take place may be an important factor in determining the pH of the displaced solution.

FLUCTUATIONS IN pH UNDER DIFFERENT IRRIGATION PRACTICES

The pH changes of soil in three plots of the experimental orchard receiving different irrigation treatments are shown in figure 2. Plot A, irrigated on a

TABLE 8
Partial analysis and pH values of water-displaced solutions of certain soils

SOIL	pH OF SOIL		MOIS- TURE PER- CENT- AGE (AIR- DRY WEIGHT BASIS)	AMOUNT AND pH OF SUCCESSIVE ALIQUOTS OF DISPLACED SOLUTION						ANALYSIS OF DISPLACED SOLUTION											
	At mois- ture equiva- lent	1-5 soil- water ratio		AIR-DRY SOIL USED	DIS- TILLED WATER ADDED TO AIR- DRY SOIL	DIS- TILLED WATER ADDED TO COM- PACTED SOIL	1		2		3		4		5		Amount of sample	Total inorganic solids	Ca	Mg	
							cc.	pH	cc.	pH	cc.	pH	cc.	pH	cc.	pH					cc.
<i>Displaced solution obtained by air pressure</i>																					
Yolo sandy loam	7.6	8.4	1.1	700	105	300	11.0	7.32	10.0	7.55	13.0	7.47	16.0	7.35	47.9	2,169	560	80.0	
Woodrow clay	...	8.9	...	1,000	180	300	18.0	8.03	24.5	8.14	23.5	8.10	19.0	8.12	35.5	8.17	
Alkaline soil from Santa Ana River flood plain	8.1	9.5	0.8	1,000	175	300	133.0	8.45	17.5	8.57	23.0	8.72	17.0	8.70	185.55	49,633	
	8.1	9.5	0.8	1,000	150	300	17.0	8.12	18.25	8.37	21.5	8.44	13.0	8.50	16.0	8.50	
Traver loam	8.1	8.6	1.2	1,000	150	300	12.0	8.30	10.5	8.34	
Traver loam	8.1	8.6	1.2	1,000	176	300	7.0	8.76	6.48	129,954	932	250.0	
<i>Displaced solution obtained by water percolation with gravity</i>																					
Yolo silty clay loam	6.9	8.0	3.5	1,700	365	500	10.0	7.42	10.0	7.54	10.0	7.53	6.75	7.55	28.0	450	88	23.0	
						500	10.0	7.57	10.0	7.61	10.0	7.64	9.00	28.4	433	94	25.0	
Ramona sandy loam	6.5	7.3	0.8	1,700	190	500	16.0	7.35	16.5	7.22	11.0	7.22	11.00	7.13	11.0	7.20	62.22	996	186	33.0	
						500	11.0	7.07	25.0	7.27	17.5	7.30	11.00	7.17	59.30	1,002	181	37.0	
Hanford sandy loam	6.7	7.2	0.6	1,700	168	500	12.5	7.09	15.0	7.00	12.5	6.97	38.82	361	109	23.0	
						500	13.0	7.12	14.0	7.07	12.0	7.00	38.35	373	101	17.0	
Altamont clay loam	7.5	8.4	6.6	1,700	500	500	12.5	7.92	9.5	8.07	14.0	7.95	14.5	7.95	26.0	7.81	49.53	406	141	11.0	
						500	9.5	7.83	
Traver loam	8.1	8.6	1.2	2,000	345	500	9.0	8.64	7.21	168,419	
						500	

2-week schedule, received 12 irrigations during 1938; plot B, irrigated whenever the top foot of soil below the mulch reached the wilting percentage, received

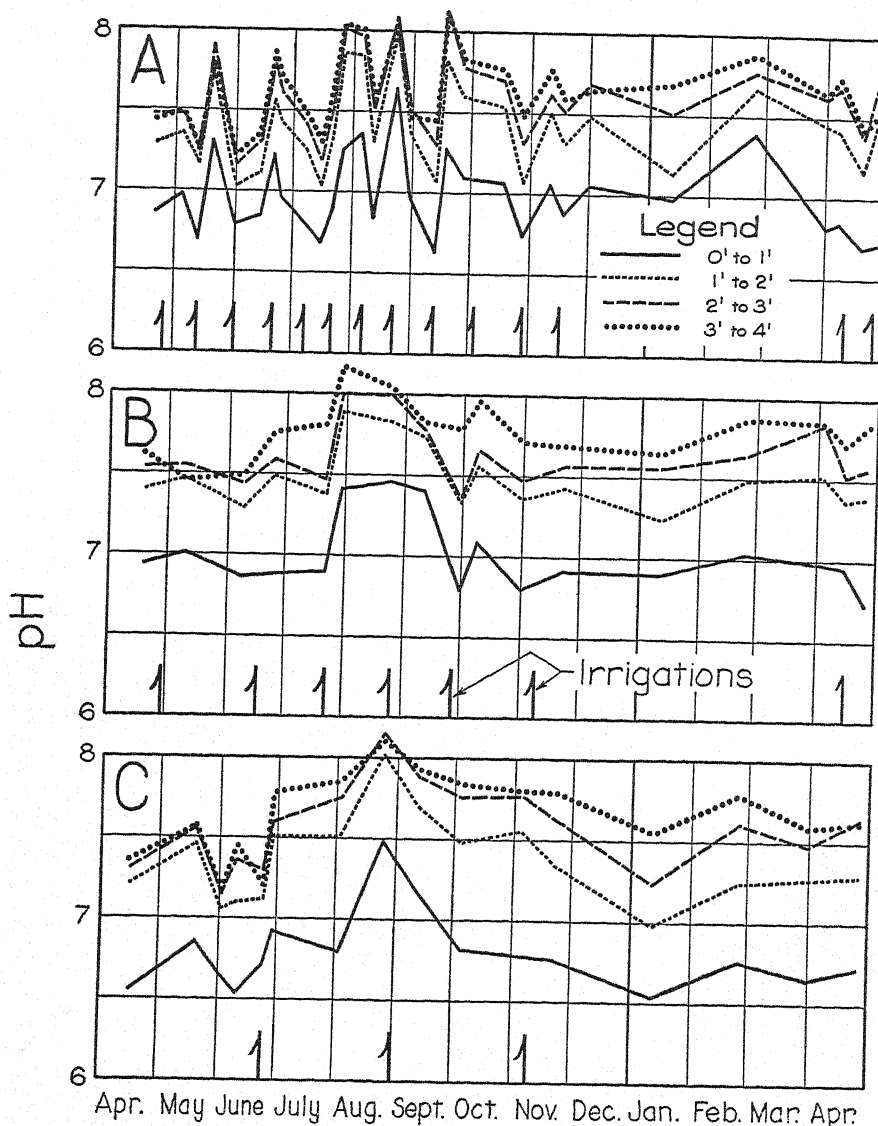


FIG. 2. SEASONAL CHANGES IN pH DURING 1938-39 IN A NAVEL ORANGE ORCHARD ON RAMONA SANDY LOAM UNDER DIFFERENT IRRIGATION TREATMENTS

During 1938, plot A received 12 irrigations; plot B, 6 irrigations; plot C, 3 irrigations

6 irrigations; and plot C, irrigated when the moisture content in the first 4 feet of soil reached the wilting percentage, received 3 irrigations. The total amounts of water, in depth, applied to the plots during the 1938 irrigation

season were: A, 33 inches; B, 27 inches; and C, 31 inches. All plots were irrigated by nine furrows placed between the tree rows, which were 24 feet apart. The soil sampling practice consisted in taking five holes uniformly spaced along a 45° diagonal on each side of a tree row; one diagonal started at the tree trunk, and the other started at the midpoint between trees in the tree row. At each successive sampling the positions of these diagonals were moved 1 foot down the tree row. Samples were taken prior to and following each irrigation.

The general trends of the curves in figure 2 are approximately the same throughout the season regardless of the irrigation practice. The higher pH values were reached during midsummer, but under the conditions of this experiment there was no net yearly change in pH. The fluctuations were most numerous and the amplitude greatest in the plot that was most frequently irrigated.

A fluctuation of approximately 0.7 in the pH of surface soil between May and November was reported by Lipman, Prince, and Blair (15). From data obtained by means of monthly soil tests in Pennsylvania, Kelley (13) concluded that there is no fixed pH value for any particular portion of soil and that the variation from the mean is not large. During the growing season the changes amounted at times to as much as one whole pH unit, in the subsoil as well as at the surface. Baver also has noted (3) that the hydrogen-ion concentration of a soil varies throughout the year. Alkaline soils showed variations in acidity ranging from 0.6–0.7 of a pH from May to September, whereas acid soils varied as much as 0.9 of a pH during this period. In acid soils in Ohio, Baver reports a continual increase in acidity from May to September with the pH returning to approximately the same value each spring. Rainfall and the season, the dehydration of silicates, or the soluble salt accumulations during the summer months are suggested as important factors affecting these variations in soil pH. Slightly alkaline plots showed no consistent pH changes.

CHANGES IN pH WITH DEPTH

For measurements of changes in pH with depth, samples were taken near a navel orange tree in a basin which was irrigated every 4 weeks. Samples from six holes were taken with a soil tube in increments of 0.2 foot to a total depth of 2 feet. Within this depth the soil was very uniform. The samples for each depth were obtained by compositing the cores from three holes. The pH values for these composited samples at the various depths are shown in figure 3. Beyond 0.4 foot there was a progressive increase in pH with depth, even though the increments in depth were small. These findings are in agreement with those shown in figure 2, where in all cases there is an increase in pH with depth.

pH OF SOIL IN SITU IN ORANGE ORCHARDS

By means of shielded extension electrodes it was possible to determine pH values of soils directly in the field. The experiments were first carried on in

certain basin-irrigated plots of the experimental orchard on one of which 50 pounds of ammonium sulfate and on the other 66 pounds of calcium nitrate had been used in 1938 as sources of nitrogen for the orange trees.

Trenches were dug to a depth of 2 feet the day following irrigation, and a large beach umbrella was placed over the trench and instruments, including all equipment necessary for determining the pH. Fresh cuts were made in the side of the trench at measured distances from the surface of the soil. A temperature reading of the soil at the desired depth was taken just prior to the making of a pH determination. The electrodes were rinsed with distilled water and wiped with fine tissue paper just before use. Soil samples were taken at the position where the pH was determined, in order to ascertain the

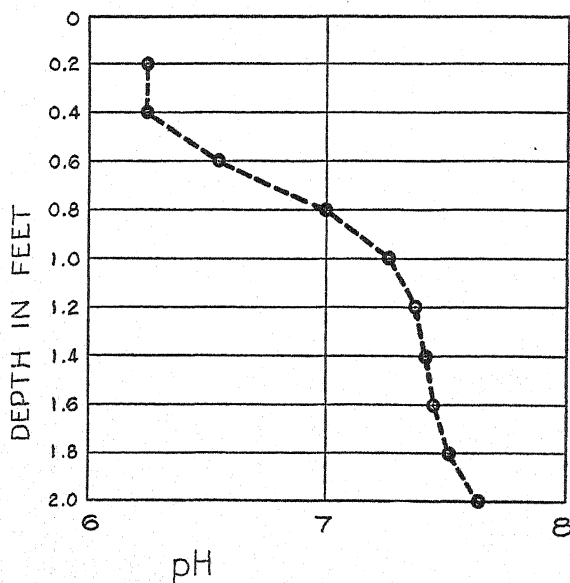


FIG. 3. CHANGES IN pH WITH DEPTH IN A BASIN-IRRIGATED NAVEL ORANGE ORCHARD ON RAMONA SANDY LOAM

moisture percentage in the soil at the various depths. Soil samples were also taken from these basin-irrigated plots with a soil tube, and composites were made of several cores. The pH values were determined for these samples after oven drying and dilution to the 1-5 soil-water ratio.

A comparison of the pH values taken *in situ* at the moisture percentages of the soil as it occurred in the orchard with the values at the 1-5 soil-water ratio (table 9) reveals that to a depth of approximately 2 feet the soil in these basins is acid at the field moisture percentage, and that the pH values at the 1-5 soil-water ratio are higher (with one exception) than those at the soil moisture percentage. When individual values or averages for the pH determinations made *in situ* are considered, those for the calcium nitrate-fertilized plot

are slightly higher than those for the ammonium sulfate-fertilized plot. When similar comparisons are made for the values at the 1-5 soil-water ratio, it is seen that the values for the calcium nitrate-fertilized plot are considerably higher than those for the ammonium sulfate-fertilized plot. The moisture percentages are high for this type of soil, since the samples were taken the day following a heavy application of water. Thus the pH values determined *in situ* are probably close to the highest values for field conditions.

It is seen in table 9 that the differences between the pH values determined at the moisture percentage of the soil and those determined at the 1-5 soil-

TABLE 9

pH Values of soil in situ in a basin-irrigated orange orchard on Ramona sandy loam and of the same soil (oven-dried at 105°C.) at 1-5 soil-water ratio

DEPTH*	SOURCES OF NITROGEN†					
	Ammonium sulfate			Calcium nitrate		
	Moisture percentage, dry soil weight basis	pH		Moisture percentage, dry soil weight basis	pH	
		<i>In situ</i>	1-5 ratio		<i>In situ</i>	1-5 ratio
<i>feet</i>						
0.1	11.9	5.07	5.03	12.6	4.98	5.31
0.3	12.0	4.30	4.87	12.3	4.40	4.95
0.5	11.3	4.52	4.85	11.6	4.63	5.45
0.7	11.8	4.84	4.93	11.0	5.20	6.64
0.9	11.7	4.97	5.35	10.9	5.10	7.00
1.1	5.12	6.74	11.3	5.42	7.07
1.3	11.7	5.47	6.75	11.5	5.50	7.05
1.5	12.2	5.54	7.00	11.0	5.72	7.22
1.7	12.5	6.08	7.10	12.1	5.78	7.35
1.9	11.3	6.07	7.15	12.2	6.20	7.54
Av.....		4.89	5.27		5.00	5.70

* Determinations of pH *in situ* were made at depths indicated in this column, whereas those on the 1-5 basis were made on soil for which the indicated depths are mean values of 0.2-foot layers. The pH of soil determined *in situ* in immediate contact with small roots ranged from 4.36 to 4.57.

† 50 pounds of ammonium sulfate or 66 pounds of calcium nitrate per tree during 1938.

water ratio may be considerable, especially in the calcium nitrate-fertilized basins. These results differ from those obtained by McGeorge (18) who compared the pH values of a group of 65 soils at the moisture equivalent and at the 1-10 soil-water ratio and found that below pH 7.0 the gap between the two curves was almost negligible until the curves reached pH 3.0. In considering differences in pH it should be remembered, as previously stated, that a difference of one unit in the range above pH 7 represents a different amount of hydrogen-ion from one unit in the range below pH 7.

The same method of determining the pH values of soil at different depths

as was used in basin-irrigated plots, was also used to obtain the pH values of soil at various depths in furrow-irrigated plots in the same orchard. A trench was cut across a furrow bottom and ridge, the same precautions being taken as those previously mentioned. The results, given in table 10, show the acid condition at the moisture percentage in the soil, the pH values ranging from 4.65 to 5.55. The average pH values (1-5 soil-water ratio) of 10 oven-dried soil samples drawn at about the same time from this same plot were 6.71 for the first foot and 7.29 for the second foot depth. Hoagland and Sharp (9)

TABLE 10
pH Values of soil in situ in a furrow-irrigated orange orchard on Ramona sandy loam

DEPTH*	pH†	
	Furrow ridge	Furrow bottom
<i>feet</i>		
0.1	4.65
0.3	5.00
0.5	5.40	5.42
0.7	5.55	5.20
0.9	5.40	5.30
1.1	5.25	5.22
1.3	5.25	5.10
1.5	5.30	5.38
1.7	5.15	5.40
1.9	5.30	5.10
2.1	4.95
2.3	5.25

* Measurements made from top of furrow ridge.

† At the 1-5 soil-water ratio, the average pH values were 6.71 for the first foot and 7.29 for the second foot depth (10 samples in each case).

TABLE 11
pH Values of unscreened calcareous soils from citrus orchards at low and high moisture contents

ORCHARD	MOISTURE PERCENTAGE, (DRY SOIL WEIGHT BASIS)	FINAL pH AFTER SUCCESSIVE MIXING OF SAME SOIL SAMPLE						pH (1-5 SOIL-WATER RATIO)
		1	2	3	4	5	6	
1	6.3	6.45	6.43	6.45	6.43	8.60
2	5.3	5.47	5.48	4.83	5.21	5.22	5.22	8.42

have shown that the pH of 1-5 soil-water suspensions of acid soils is not markedly affected by increasing the content of CO₂ up to 10 per cent and that the pH values of slightly alkaline soils are only slightly increased by such treatments (the mean CO₂ content of arable soils being taken as 0.25 per cent).

SOIL MOISTURE EFFECTS ON pH OF CALCAREOUS SOILS

Table 11 gives the results obtained with highly calcareous soils taken in citrus areas where chlorosis frequently is marked. One soil with a moisture

percentage of 6.3 gave a pH value of 6.43, whereas at the 1-5 soil-water ratio a pH of 8.60 was obtained. A second soil at a moisture percentage of 5.3 yielded a pH value of 5.22, whereas at the 1-5 soil-water ratio the pH was 8.42. Table 11 shows the final pH readings on the same samples of soil after they were repeatedly stirred between successive series of readings in obtaining the final equilibrium values. The marked changes in pH at the low and high dilutions, give some idea of the large changes that may occur in the pH of calcareous soils in which citrus trees are grown. Successive determinations were made at these low moisture contents in order to determine the degree of reproducibility of the results without undue precautions being taken. Fairly uniform results may be had by thorough mixing and by macerating the soil free of lumps with a stout stirring rod, care being taken that temperature effects are compensated for.

In 1935 McGeorge (16) suggested that the determination of the pH value of basic soils be made four times on each soil: 1 and 2, 1 to 10 soil-water suspension within 2 hours and after standing 24 hours; 3, 1 to 1 soil-water suspension; and 4, 1 to 10 soil-water suspension plus 10 cc. each of 2 *M* NaCl and 2 *M* CaCl₂. In 1938 as a result of further investigation (18) the moisture equivalent of soils was given added importance in the determination of the pH values. These and our results are in decided contrast with the findings of Sharp and Hoagland (24), who concluded that the pH values of soil suspensions approximate those of the soil solutions.

The recent impetus given to the study of pH in soils and the effects of dilution and hydrolysis on the pH values obtained, may cause one to overlook giving proper credit to Salter and Morgan (23), whose conclusions are mentioned by McGeorge (16). Although it was recognized that no direct measurement of pH could be obtained by the sucrose hydrolysis method, nevertheless with soils at moisture contents varying from high dilutions to those approaching the soil moisture contents found under field conditions, Salter and Morgan were able to use the velocity of sucrose hydrolysis as an index of relative reaction. Their results for a wide range of dilution show that as the dilution increased, the pH increased. Notwithstanding the fact that the pH values calculated for the low moisture contents were much lower than those at the 1-5 soil-water ratio, they recommended the 1-5 ratio because of its greater suitability of both colorimetric and electrometric procedures. They suggested that, for a more accurate idea of the reaction at low moisture percentages, the graph connecting the pH determined at the three soil-water ratios, preferably 1 to 5, 1 to 25, and 1 to 125, be extrapolated. Reference to figure 1, in the present investigation, indicates that such a procedure would most likely lead to gross error. Recently Itano and Tuzi (10) tested the applicability of the quinhydrone electrode under natural field conditions in soils of varying moisture content. The pH measurements could be carried on with soils containing as low as 5 per cent moisture. The pH values under natural conditions were 0.5-0.9 unit lower than the values obtained in the laboratory by

the usual procedures. Aoki (1) found no regular relation between pH and the moisture content of soil by the method used by the aforementioned investigators (10).

Rainfall as a factor in the pH of orchard soil

As previously mentioned, rainfall has been suggested (3) as a possible factor in seasonal changes in the pH of soils. In order to determine the effectiveness of a given amount and the time distribution of rainfall, a large canvas was used to cover a plot of soil in the furrow-irrigated orchard. The canvas covered an area of soil in the tree row equal to that covered between the tree rows. It extended from a tree to half the distance to the next tree in the tree row, and to 12 feet on each side of a tree row. When it was not raining, the canvas was removed, but during a rain it was put in position on stakes in order to raise it off the ground for purposes of ventilation. The experiment was begun December 3, 1938, prior to the seasonal rainfall, and ended April 19, 1939. The rainfall distribution was as follows: December 15-21, 4.44 inches; January 3-February 1, 2.37 inches; February 1-20, 1.67 inches; March 9-28, 1.20 inches; and April 2-14, 0.29 inch. The precipitation during the experimental period was approximately equal to that of a normal year.

The pH of rain was also determined at various times during the experimental period, as follows: December 14-15, 0.18 inch rain, pH 6.93; December 16, 0.48 inch, pH 6.92; December 18, 0.70 inch, pH 6.59; December 19, 1.42 inches, pH 6.73; March 9, 0.38 inch, pH 6.73. The pH of distilled water was 5.13.

Table 12 gives the results obtained in the rain-protected and the unprotected area. Comparisons of the average pH values for the 4-foot depths for any given sampling data indicate that the rains had no effect on the pH of the soil as determined at the 1-5 soil-water ratio, either in the plot in the tree row or in that between the tree rows. For the samples collected on April 11, 1939, the pH values were determined not only at the 1-5 soil-water ratio but also at the moisture content of the soil at the time of sampling. In the samples taken in the tree rows the average pH values for April 11 by the two methods of preparing the samples differed by approximately 1 pH unit, whereas in those taken in the plots between the tree rows, the values by the two methods of preparing the samples differed by about $1\frac{1}{4}$ pH units.

EFFECT OF LEACHING BY IRRIGATION ON pH OF SOIL

The basin-irrigated plots of the orange orchard previously mentioned were utilized to determine the effect of leaching on the pH of soil. One group of plots received 4 inches of irrigation every 2 weeks, and the other lots were irrigated on a monthly schedule. There was also a difference with respect to the fertilization practice during 1938, as follows: A, no fertilizer; B, 50 pounds of ammonium sulfate per tree per annum; C, 66 pounds of calcium nitrate per tree per annum. Soil samples were taken to a depth of 12 feet,

TABLE 12
pH Values of soil (1-5 soil-water ratio) from covered and uncovered orchard plots, Ramona sandy loam

DEPTH	12/3/38	1/12/39	1/28/39	3/17/39	4/11/39*	AVERAGE MOISTURE CONTENT 4/11/39	4/11/39	4/19/39	4/11/39	4/19/39
feet	pH	pH	pH	pH	pH	per cent	pH	pH	pH	pH
<i>Plot in tree row (rain proofed) not irrigated or fertilized</i>										
1	7.11	7.09	7.14	7.22	5.88	4.6	7.04	7.19		6.30
2	7.39	7.25	7.57	7.59	6.27	6.0	7.34	7.60		7.26
3	7.52	7.48	7.78	7.80	6.50	7.4	7.52	7.72		7.52
4	7.64	7.55	7.92	7.93	6.63	9.2	7.78	7.80		7.59
Av.....	7.37	7.30	7.49	7.54	6.22		7.33	7.51		6.81
<i>Plot between tree rows (rain proofed) irrigated and fertilized</i>										
	6.65	6.62	7.06	6.57	5.48	4.5	6.65	6.30		6.30
	7.40	7.39	7.69	7.42	5.89	6.4	7.35	7.26		7.26
	7.66	7.65	7.98	7.76	6.48	8.5	7.58	7.52		7.52
	7.60	7.64	7.99	7.86	6.49	7.4	7.66	7.59		7.59
	7.11	7.10	7.50	7.08	5.88		7.10	6.81		6.81
<i>Plot between tree rows (not rain proofed) irrigated and fertilized</i>										
	6.73	6.82	7.32	6.38	5.45	5.1	6.67	7.00		7.00
	7.04	7.27	7.75	7.40	5.71	5.7	7.07	7.41		7.41
	7.35	7.50	7.98	7.72	6.10	7.3	7.50	7.67		7.67
	7.74	7.56	8.00	7.85	6.31	7.1	7.62	7.73		7.73
	7.07	7.18	7.67	6.91	5.77		7.06	7.35		7.35
<i>Plot in tree row (not rain proofed) not irrigated or fertilized</i>										
1	7.09	7.10	7.34	7.08	6.16	5.9	7.00	7.05		7.05
2	7.19	7.26	7.66	7.52	6.40	7.5	7.15	7.42		7.42
3	7.40	7.45	7.87	7.77	6.47	8.0	7.58	7.66		7.66
4	7.56	7.53	8.04	7.85	6.75	10.2	7.79	7.71		7.71
Av.....	7.27	7.30	7.64	7.44	6.40		7.27	7.37		7.37

* pH determinations at the moisture content (dry soil weight basis) of the samples, as shown in column to the right.

and pH determinations were made at the 1-5 soil-water ratio. Soils in plots receiving the heavier applications of water (8 inches every month) showed a lower pH than those receiving 4 inches every 4 weeks (table 13).

RELATION OF FERTILIZATION PRACTICE TO SOIL pH

The question of whether the pH of soil in the same group of plots as those referred to in table 13 was affected by the use of certain fertilization practices was considered. Table 14 shows the pH values obtained at the sample moisture content and at the 1-5 soil-water ratio of soil samples drawn April 11, 1939. In both cases the pH values of samples from the ammonium sulfate-fertilized basins were lower in the first and second, and higher in the third

TABLE 13

pH Values of soil samples (1-5 soil-water ratio) taken to a depth of 12 feet in a basin-irrigated orange orchard on Ramona sandy loam under various irrigation and fertilizer treatments

TREATMENT*	1 FT.	2 FT.	3 FT.	4 FT.	5 FT.	6 FT.	7 FT.	8 FT.	9 FT.	10 FT.	11 FT.	12 FT.
A	7.47	7.65	7.77	7.57	7.50	7.48	7.51	7.18	7.04	7.22	7.52	7.90
A	7.27	7.55	7.80	7.80	7.52	7.67	8.55	8.22	7.00	7.94	7.92	8.07
B	5.30	7.30	7.68	8.24	8.17	8.40	8.58	8.57	7.00	7.28	6.98	6.96
C	6.98	7.72	7.88	7.91	8.41	8.69	8.72	8.93	8.85	8.75	8.32	9.15
C	7.32	7.90	7.94	7.66	8.52	8.61	8.70	8.67	8.72	9.40	9.52	9.32
D	6.94	6.80	7.64	7.65	8.42	8.67	8.73	8.84	8.95	9.24	9.27	9.41
D	7.27	7.81	7.92	8.31	8.49	8.33	8.84	9.25	9.43	9.30	9.25	9.47
E	7.27	8.10	8.00	8.01	8.50	8.70	8.68	8.85	8.84	8.90	8.24	8.27
A, A, B, (Av.)...	5.78	7.47	7.74	7.79	7.64	7.71	7.91	7.61	7.01	7.39	7.31	7.37
D, E, (Av.)....	7.13	7.20	7.83	7.91	8.47	8.54	8.74	8.94	9.01	9.11	8.64	8.69

* Fertilizer treatment of tree squares: A, no fertilizer since March, 1937; B, 50 pounds ammonium sulfate during 1938; C, 66 pounds calcium nitrate during 1938; D and E, 5 pounds of ammonium sulfate per annum.

Seasonal irrigation practice: Plots A, B, and C receive an average depth of water of 4 inches every 2 weeks; plots D and E receive 4 inches once a month.

and fourth foot samples than in samples from corresponding depths in the calcium nitrate-fertilized basins. The pH values, at the soil moisture content in the field, were highest in the samples from the unfertilized basins. At the 1-5 soil-water ratio, the pH value of the first foot of plot A was markedly higher than those of plots B and C.

Determinations of pH were made also at the 1-5 soil-water ratio of samples taken May 12, 1939 and December 3, 1938, at increments of 0.2 feet to a depth of 2 feet. The average pH values for the first foot depth were 7.05, 6.51, 4.94, and 5.71, and for the second foot depth, 7.40, 7.47, 6.85, and 7.42 for plots A, B (December 3), B, and C, respectively. Ammonium sulfate markedly affected the average pH values of the first and second foot samples.

The average pH values of the May 12, 1939, samples taken after the spring application of ammonium sulfate are lower than those of the December 3, 1938, samples. The average pH values of the samples taken May 12, 1939, from the calcium nitrate-treated plot are higher than those of the samples taken on the same date from the ammonium sulfate-treated plot.

TABLE 14

*pH Values of soil in a basin-irrigated orange orchard on Ramona sandy loam fertilized with various sources of nitrogen**

DEPTH	pH (1-5 SOIL-WATER RATIO) MAY 12, 1939				DEPTH	MOISTURE PER- CENTAGE (DRY SOIL WEIGHT BASIS)			pH (APRIL 11, 1939)†					
	A	B‡	B	C		A	B	C	At sample moisture			1-5 soil-water ratio		
<i>feet</i>					<i>feet</i>				A	B	C	A	B	C
0 -0.2	7.30	6.25	5.20	6.07										
0.2-0.4	7.00	6.25	4.83	5.15	1	5.9	8.1	6.8	6.49	4.02	5.55	7.06	5.00	6.64
0.4-0.6	6.93	6.55	4.78	5.78										
0.6-0.8	7.00	7.00	4.77	6.67	2	8.2	9.6	9.2	7.03	6.15	6.75	7.48	7.02	7.46
0.8-1.0	7.12	7.26	5.37	7.20										
0 -1.0(Av.)...	7.05	6.51	4.94	5.71										
1.0-1.2	7.35	7.37	6.48	7.36	3	9.6	11.1	9.7	7.14	6.90	6.57	7.68	7.67	7.37
1.2-1.4	7.63	7.43	6.81	7.34										
1.4-1.6	7.20	7.45	7.00	7.40	4	8.9	12.5	11.4	7.14	6.91	6.52	7.75	8.28	7.41
1.6-1.8	7.35	7.52	7.15	7.47										
1.8-2.0	7.60	7.64	7.23	7.63										
1.0-2.0(Av.)...	7.40	7.47	6.85	7.42										
0 -2.0(Av.)...	7.19	6.76	5.22	6.00										

* Fertilizer treatment of tree squares: A, no fertilizer since March, 1937; B, 50 pounds ammonium sulfate during 1938; C, 66 pounds calcium nitrate during 1938. The amounts of fertilizer applied to B and C are much higher than are used commercially.

† Average of four separate samples.

‡ Samples taken December 3, 1938.

SUMMARY

An orange orchard on Ramona sandy loam, in which various irrigation practices were followed, served as a source of soil samples for the determination of pH. Oven-dried samples taken for soil moisture determinations thus were available for pH studies. In addition, many soil samples were drawn specifically for pH determinations, and finally pH values were obtained directly in the orchard with soils *in situ*. The titration and spear-type glass electrodes and in some cases the extension-type shielded glass electrode were employed, both the Coleman and Beckman instruments being available.

The pH values of air-dried samples (1-5 soil-water ratio) from the orchard were consistently higher than those of oven-dried samples.

Prolonged heating of Aiken clay loam and of Ramona sandy loam lowered the pH at both the 1-5 and 1-10 soil-water ratios, but did not change the pH of Tujunga sand and of Traver clay.

Storage of oven-dried samples of the orchard soil in paper bags was accompanied by an increase in pH (1-5 soil-water ratio); oven drying of these samples gave pH values very similar to those of the original oven-dried samples. The pH of air-dried samples varied. Air-dried samples which showed an increase in pH upon storage were returned to approximately their original pH upon being oven dried.

The size of samples of the Aiken, Ramona, Tujunga, and Traver soils, tested at the 1-5 soil-water ratio, had no apparent effect on pH.

For any given dilution or size of sample tested, the Ramona, and Traver soils showed an increase in pH as the dilution of the sample increased.

The pH of the solution displaced by gravity from Yolo sandy loam was approximately that found at the moisture equivalent, whereas the pH values of the displaced solutions of Ramona sandy loam and Hanford sandy loam were closer to those determined in soil suspensions at the 1-5 soil-water ratio. The pH of the solution displaced by air pressure from Traver loam was higher at the higher soil moisture content.

Fluctuations in pH values of soil in the orchard under different irrigation practices were followed from the spring of 1938 to that of 1939. The seasonal trends of the curves for pH (1-5 soil-water ratio) are approximately the same regardless of whether the plots were irrigated every 2 weeks during the season (12 irrigations), whenever the first foot of soil below the mulch reached the wilting percentage (6 irrigations), or when the wilting percentage was reached in the first 4 feet of soil (3 irrigations). The highest pH values were reached during midsummer. Under the conditions, there was no net yearly change in pH. Fluctuations were more numerous and the amplitude greatest in the most frequently irrigated plot. The curves for the changes of pH with depth throughout the season are approximately parallel for any one of the practices studied.

The pH values (1-5 soil-water ratio) of a uniform soil sample from the orchard showed a progressive increase from 0.4 foot to a depth of 2 feet with increments in depth of 0.2 foot only.

The pH of soil was determined *in situ* in basin- and in furrow-irrigated plots that received ammonium sulfate or calcium nitrate as a source of nitrogen. At the field moisture capacity the soil samples from the basin-irrigated plots were acid to a depth of about 2 feet and had lower pH values than the samples determined at the 1-5 soil-water ratio. When determined *in situ*, the pH values of the basin-irrigated soil in the calcium nitrate-fertilized plot were slightly higher than those of soil in the ammonium sulfate-fertilized plot. The pH determinations at the 1-5 soil-water ratio were higher for the calcium-

nitrate than for the ammonium sulfate-fertilized plot. Since the determinations were made the day following irrigation, the values obtained for the soil *in situ* were probably close to the highest for field conditions. The difference between the pH values at the field moisture percentage and at the 1-5 soil-water ratio is considerable in many instances, especially when calcium nitrate is used as the source of nitrogen. In the furrow-irrigated plot, at the field-moisture percentage, the pH values ranged from 4.65 to 5.55 in the first 2 feet of depth. At the 1-5 soil-water ratio the average pH values were 6.71 for the first foot and 7.29 for the second foot depth.

The soil moisture percentage is of considerable significance in the pH of calcareous citrus soils. One soil with a moisture percentage of 6.3 gave a pH value of 6.43, whereas at the 1-5 soil-water ratio a pH of 8.60 was obtained. A second soil with a moisture percentage of 5.3 gave a pH value of 5.22, whereas at the 1-5 soil-water ratio the pH was 8.42.

Rainfall, in an amount equal to that of a normal year at Riverside, had no effect on the pH of the soil (as determined at the 1-5 soil-water ratio) either in tree rows or between tree rows. The pH values of the samples at the field moisture percentages were lower, in general, between the tree rows (5.88 for rain-protected and 5.77 for unprotected plots) than in the plots in the tree rows (6.22 for rain-protected and 6.40 for unprotected plots). The soils at the field-moisture percentages were acid in reaction.

The effect of leaching on the pH of soil in the orchard to a depth of 12 feet was investigated. An average depth of 4 inches of water was applied to certain basin plots every 2 weeks and to other basin plots once a month. The pH values obtained in the plots under the 2-week schedule, where a considerable amount of water moved through the soil, were lower than those irrigated on a monthly schedule.

REFERENCES

- (1) AOKI, M. 1938 Determination of soil pH under natural field conditions. *Jour. Agr. Chem. Soc. Japan* 14: 165-177.
- (2) BAILEY, E. H. 1932 The effect of air drying on the hydrogen-ion concentration of the soils of the United States and Canada. U. S. Dept. Agr. Tech. Bul. 291.
- (3) BAVER, L. D. 1927 Factors affecting the hydrogen-ion concentration of soils. *Soil Sci.* 23: 399-414.
- (4) BUEHRER, T. F., AND WILLIAMS, J. A. 1936 The hydrolysis of calcium carbonate and its relation to the alkalinity of calcareous soils. *Ariz. Agr. Exp. Sta. Tech. Bul.* 64.
- (5) BURD, J. S., AND MARTIN, J. C. 1923 Water displacement of soils and the soil solution. *Jour. Agr. Sci.* 13: 265-295.
- (6) DEAN, H. L., AND WALKER, R. H. 1935 A comparison of glass and quinhydrone electrodes for determining the pH of some Iowa soils: III. The change in pH of the soil-water mixture with time. *Jour. Amer. Soc. Agron.* 27: 585-595.
- (7) HAAS, A. R. C. 1938 pH for healthy growth in citrus. *Calif. Citrogr.* 23: 158, 176, 178, 180, 181.
- (8) HAAS, A. R. C. 1939 Growth of citrus and walnut trees as affected by pH: I, II, III. *Calif. Citrogr.* 24: 351, 364, 379; 388, 406, 407, 408; 430, 458.

- (9) HOAGLAND, D. R., AND SHARP, L. T. 1918 Relation of carbon dioxide to soil reaction as measured by the hydrogen electrode. *Jour. Agr. Res.* 12: 139-148.
- (10) ITANO, A., AND TUZI, Y. 1938 Direct pH determination of soil under its natural state by the quinhydrone method: III. Determination of pH of soils in the dry form. *Ber. Ohara Inst. Landw. Forsch.* 8: 83-95.
- (11) JOSEPH, A. F., AND MARTIN, F. J. 1923 The hydrogen-ion concentration of heavy alkaline soils. *Jour. Agr. Sci.* 13: 321-332.
- (12) KEATON, C. M. 1938 A theory explaining the relation of soil-water ratios to pH values. *Soil Sci.* 46: 259-265.
- (13) KELLEY, A. P. 1923 Soil acidity, an ecological factor. *Soil Sci.* 16: 41-54.
- (14) KELLEY, W. P., AND BROWN, S. M. 1921 The solubility of anions in alkali soils. *Soil Sci.* 12: 261-285.
- (15) LIPMAN, J. G., PRINCE, A. L., AND BLAIR, A. W. 1921 The influence of varying amounts of sulfur in the soil, on crop yields, hydrogen-ion concentration, lime requirement and nitrate formation. *Soil Sci.* 12: 197-207.
- (16) McGEORGE, W. T. 1935 The measurement and significance of hydroxyl-ion concentration in alkaline-calcareous soils. *Ariz. Agr. Exp. Sta. Tech. Bul.* 57: 239-271.
- (17) McGEORGE, W. T. 1937 The determination of soil reaction under field conditions by means of the spear-type glass electrode. *Jour. Amer. Soc. Agron.* 29: 841-844.
- (18) McGEORGE, W. T. 1938 Factors contributing to the reaction of soils and their pH measurement. *Ariz. Agr. Exp. Sta. Tech. Bul.* 78.
- (19) McGEORGE, W. T. 1938 Report on hydrogen-ion concentration of soils of arid and semi-arid regions. *Jour. Assoc. Off. Agr. Chem.* 21: 246-247.
- (20) PIERRE, W. H. 1925 The hydrogen-ion concentration of soils as affected by carbonic acid and the soil-water ratio and the nature of soil acidity as revealed by these studies. *Soil Sci.* 20: 285-305.
- (21) PLUMMER, J. K. 1918 Studies on soil reaction as indicated by the hydrogen electrode. *Jour. Agr. Res.* 12: 19-31.
- (22) PURI, A. N., AND ASGHAR, A. G. 1938 Influence of salts and soil-water ratio on pH value of soils. *Soil Sci.* 46: 249-257.
- (23) SALTER, R. M., AND MORGAN, M. F. 1923 Factors affecting soil reaction: I. The soil-water ratio. *Jour. Phys. Chem.* 27: 117-141.
- (24) SHARP, L. T., AND HOAGLAND, D. R. 1916 Acidity and absorption in soils as measured by the hydrogen electrode. *Jour. Agr. Res.* 7: 123-145.
- (25) WILCOX, J. C. 1936 Some factors involved in the colorimetric determination of the pH of soils. *Sci. Agr.* 16: 225-232.

CONSISTENCY AND PHYSICOCHEMICAL DATA OF A LOESS PAMPANEO SOIL: II. PROPERTIES OF NATURAL AND HOMOIONIC SAMPLES OF SOILS AND CLAYS

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It is the purpose of this paper to adduce data which are of help in defining more clearly the physical and chemical factors that influence the consistency and water-retaining properties of soils.

The desirability of such an undertaking is evident from the fact that, though testing methods for the determination of those properties are widely used and have been thoroughly standardized, little is known about the physical and chemical factors which govern the properties measured by these methods. Obviously, an understanding of these factors must be the basis for the logical interpretation and the best possible usage of the test data. The little progress which has been made in this direction is due, in part, to the complexity of the problem itself; in part, to the fact that the profession which has the greatest practical interest in consistency properties at the present time (highway engineering) does not, in general, possess the scientific tools for the solution of this problem.

The complexity of the problem is a result of the multiphase character of any soil-water system and the predominance of secondary influences which are typical for the system, as such, but not for its various components. In a preceding paper (12) on the same general topic, it was shown that the consistency properties of soil samples taken from different depths of the same profile depended not only on the relative amounts of clay and colloids present, but also on the content of silt and organic matter. Other, more essentially chemical, influences, such as that of the C/N ratio of the organic matter were indicated although not demonstrated in detail.

SOIL MATERIALS USED

The soil materials used were the samples of topsoil and subsoil of loess pampaneo previously described (12). Three variations of these materials

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were tested; namely, the natural soils in the condition as received; homoionic variations of the soils, prepared by percolating salt solutions through soil samples and washing out excess salt as well as possible; homoionic clays, prepared by extraction of the clay (ultracentrifuge), electrodialysis, and neutralization, with respective hydroxides.

PROPERTIES DETERMINED AND METHODS USED

The properties determined on the soils and their homoionic variations were (a) the standard tests recommended for subgrade soils by the U. S. Public Roads Administration (4)—liquid limit, plastic limit, field moisture equivalent, etc.; (b) heat of wetting by means of a water calorimeter as described in part I (12); (c) sorption of water and other liquids by means of the Winterkorn-Baver apparatus (11); (d) slaking resistance, according to the Russian method. Soil specimens, formed in the mold generally used for preparation

TABLE 1

Results of routine subgrade soil tests

SOIL	LOWER LIQUID LIMIT*	LOWER PLASTIC LIMIT*	PLASTIC INDEX*	VOLUME CHANGE AT FIELD MOISTURE EQUIVALENT*	SHRINKAGE LIMIT*	SHRINKAGE RATIO*	FIELD MOISTURE EQUIVALENT*	MOISTURE EQUIVALENT (VACUUM)*	MECHANICAL ANALYSIS†			
									Passing # 200 sieve	Silt (diam. 0.05-0.005 mm.)	Clay (diam. 0.005 mm.)	Colloids (diam. 0.001 mm.)
Topsoil	38.4	28.6	9.8	14.7	24.0	1.48	33.9	29.9	95.8	51.0	29.0	13.0
Subsoil	60.7	27.4	33.3	46.3	14.4	1.89	38.9	55.8	98.2	33.0	53.0	34.0

* Results on material passing #40 sieve.

† In per cent.

of tensile strength samples of cement mortar, are coated with paraffin on both ends, leaving an uncoated center strip $\frac{1}{4}$ -inch wide around the neck. These specimens are suspended in water, and the time of separation of the lower from the upper part of the specimen is noted as slaking time (10); (e) the Waksman method of analysis of the organic matter (8).

The properties determined on the extracted clays were silica-sesquioxide ratio, base-exchange capacity, and sorption of water and other liquids.

DISCUSSION OF DATA OBTAINED

Change of the soil constants as a function of the exchange ions

Table 1 contains the results of the routine tests performed on the topsoil and the subsoil samples. Figure 1 demonstrates how the physical properties tested change by substitution of cations for those originally adsorbed on the internal soil surface. This influence also extends to the mechanical analysis

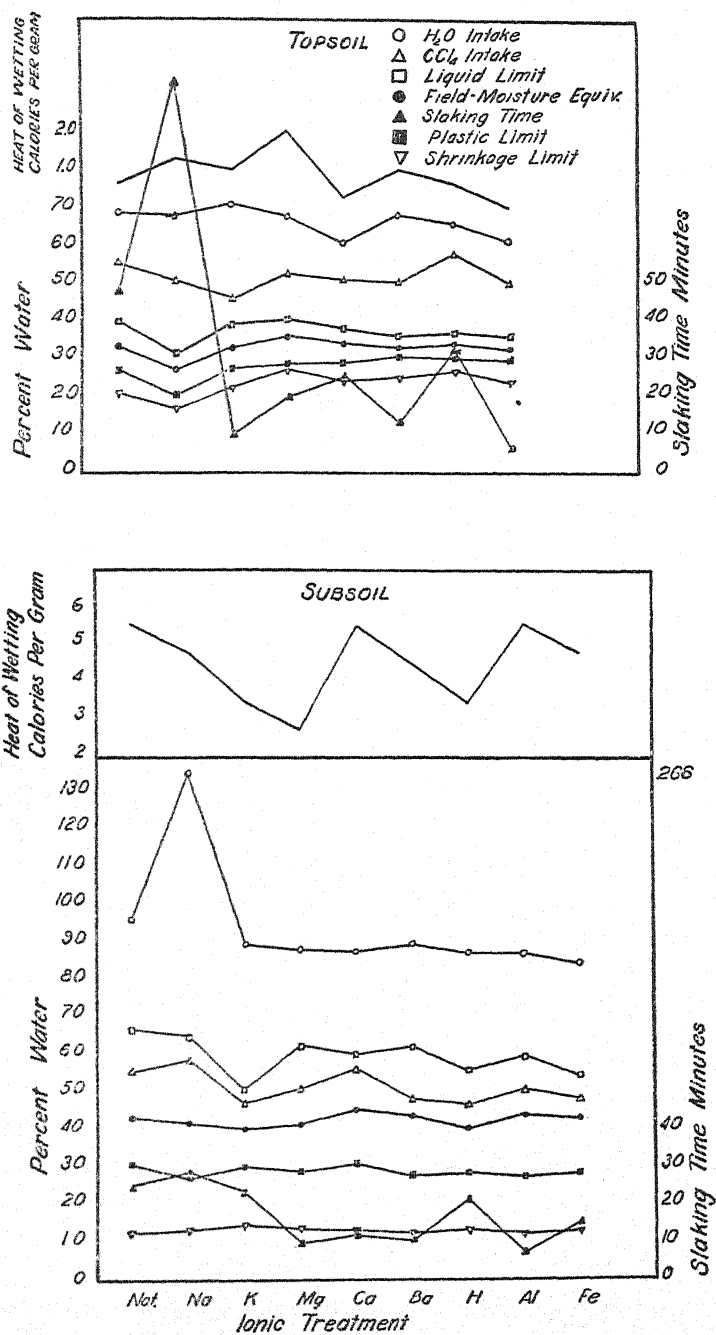


FIG. 1. INFLUENCE OF IONIC SUBSTITUTION ON CONSISTENCY PROPERTIES AND SORPTIVE BEHAVIOR OF TOPSOIL AND SUBSOIL

of homoionic soils, where changes in apparent particle size are caused by ionic substitution.

The volume elasticity of a soil is an important property in regard to its use in highway construction. An indicator of this property in dry condition is the shrinkage limit; in wet condition, the liquid limit.

As can be seen in figure 1, the shrinkage limit is a function of the exchange ions. According to the data, the dry elasticity of the topsoil (shrinkage limit 21.0) can be reduced by treatment with Na ion (shrinkage limit 17.0) and increased by treatment with all the other ions used. In the subsoil, all ionic treatments tend to increase the elasticity, although not to the same extent as in the topsoil (shrinkage limits from 12.8 to 14.9).

The liquid limit is a function of both the water affinity of the internal soil surface and the elasticity of the soil skeleton. It is somewhat difficult to separate these two influences. The reduction of the liquid limit of the natural topsoil (40.1) by Na-ion substitution (31.7) is, however, significant. In the less elastic subsoil the influence of ionic substitution on the water affinity of the soil overshadows that on the elasticity.

TABLE 2
Effect of exchange cations on plastic index

ION.....	NAT.*	Na	K	Mg	Ca	Ba	H	Al	Fe
Topsoil.....	12.9	11.5		11.8	12.0	9.1	5.7	6.8	5.8
Subsoil.....	36.3	37.3	20.2	33.4	29.1	34.3	27.1	32.0	25.8

* Ionic saturation of natural soil: 24.2 per cent Na; 47.1 per cent Ca; 17.6 per cent Mg.

The consistency properties of soils can be viewed as expressions of their internal friction and cohesion. The cohesion of soils has been found to increase with an increase of their plastic index: therefore, the effect of the exchange ions on this property is important. As can be seen from table 2, the cohesive properties of the topsoil and of the subsoil can be appreciably reduced by treatment with hydrogen and trivalent ions. The great reducing effect of the potassium ion in the case of the subsoil is especially remarkable.

Kögler (5, p. 60) has shown a general inverse correlation between the plastic index and the angle of internal friction of cohesive soils. According to his generalization, the topsoil would have an angle of friction of about 28° , which can be changed into one of about 31° by treatment with hydrogen ions. The subsoil would have an angle of about 20° , which is changed into one of 25° by treatment with potassium ions. Although the terms "friction" and "cohesion" do not possess as definite a physical meaning as would be desirable, nevertheless this consideration helps to point out the marked effect of ionic substitution on the engineering properties of soils.

As can be seen from figure 1, the curves showing the functional connection between the soil constants and the exchange ions for the topsoil follow rather

closely the pattern set by the shrinkage limit. This indicates that the elasticity of this soil is its dominating characteristic. Because of the loose bedding of the soil particles in the determination of the liquid intake by means of the Winterkorn-Baver method, the elasticity of the soil is not evidenced very much in the data bearing on this phase. Though the curve representing the intake of CCl_4 by the topsoil is rather difficult to explain, the relationship between the heat of wetting and the H_2O intake is obvious, especially in the case of the Ba, H, Al, and Fe ions, which show predominantly hydration-swelling. The other part of the curve appears to indicate osmotic-swelling effects (9).

The slaking time of soils is a complex function of the size of the individual soil pores, the energy of wetting, the swelling capacity, the total pore space, and the viscosity of the water. As a result, the slaking curve of the homoionic modifications of the topsoil cannot be correlated with the curves indicating the other soil properties. The effect of the Na ion in increasing the slaking resistance, however, deserves comment.

In the case of the topsoil the elasticity appeared to be the dominant factor; in the case of the subsoil, the energy of wetting appears to be the most influential item. Its influence, however, is found only by a relatively close study of the curves in figure 1.

The curve for the heat of wetting of the homoionic modifications of the subsoil, that for the field moisture equivalents, and, in the main, that for the liquid limits are of the same general type; in the last only the Ca-soil falls somewhat out of the general order. The curve for the slaking data appears to be, in the main, an inverse function of the liquid limit curve, only the natural and Na-soils representing major exceptions. There appears to be little reason for doubt that these exceptions are caused by the osmotic type of swelling which is marked in these soils.

Composition of the organic matter in the soil

Because of its influence on the physical and chemical reactions of soils (1, 2), the content and the approximate composition of the organic matter in the topsoil and subsoil were determined.

Briefly, the essential features of the method are as follows: ether extraction in Soxhlet apparatus for 12–24 hours; extraction with 95 per cent ethyl alcohol for 1–2 hours; hot-water extraction; extraction with 2 per cent HCl for 5 hours at 100°C .; treatment of 20 gm. of the residue with 20–30 cc. of 80 per cent H_2SO_4 for $2\frac{1}{2}$ hours in the cold, followed by dilution with 15 volumes of water and boiling for 2–5 hours. The residue is washed and dried.

These data can be considered from a pedological viewpoint by their correlation with results of analysis by other investigators. The organic matter in the chernozem type of soils is believed to have been subjected to more decomposition than the organic matter of the podzols. This advanced state of decomposition is manifested by a lower cellulose and hemicellulose content

and a higher protein content. An examination of the published data on the extraction analysis of chernozem soils shows that the topsoil has generally a higher amount of hemicellulose and cellulose than the subsoil, indicating more decomposition in the lower horizon. The Argentina topsoil and subsoil showed only traces of hemicellulose, indicating an advanced stage of decomposition. The protein content of various chernozem soils, as reported in the literature, is higher in the topsoil in some cases and lower in other cases. In the Argentina soil, the protein content was slightly higher in the topsoil than in the subsoil, with the protein content relatively high in both cases. The protein content of the Argentina soil is higher than that reported by Waksman and Hutchings (7) for the chestnut soils and lower than that of the serozem soils, indicating a stage of decomposition intermediate to these two soil types.

Published data on the water-soluble constituents of soil-organic matter (6) indicate that under conditions of a movement of water downward through the

TABLE 3
Nature of the organic matter in loess pampeano soils

CONSTITUENT	PER CENT OF TOTAL ORGANIC MATTER		PER CENT OF AIR-DRIED SOIL		
	Topsoil	Subsoil	Topsoil	Subsoil	Difference
Total organic matter.....	4.5	1.95			
Protein.....	47.00	42.75	1.615	0.833	0.782
Lignin.....	37.40	42.05	1.588	0.625	0.963
Celluloses.....	4.8	9.6	1.96	1.87	0.09
Hemicelluloses.....	5.8	10.4	0.261	0.202	0.059
Sugars, amino-acids, etc.....	2.88	1.52	0.129	0.029	0.100
Wax, resins, alkaloids, etc.....	0.89	2.11	0.040	0.041	
Wax, resin-like fatty substances.....	1.20	1.57	0.054	0.030	0.024

profile, the percentage of the organic matter soluble in water is higher in the subsoil than in the topsoil. Under conditions in which the rainfall approximates the evaporation, the amount of water-soluble material of the organic matter is about the same in the topsoil and in the subsoil. Under conditions where the evaporation is higher than the rainfall, the water-soluble portion of the organic matter is higher in the topsoil than in the subsoil. Since these observations are made on a limited number of soils, they remain to be confirmed by further investigations. The inferences made, however, appear to be logical.

The foregoing observations on the organic matter are helpful in typing the Argentina soil as to its pedological features. This soil has evidently been subjected to a type of weathering intermediate to those responsible for the formation of the serozem soils and of the chestnut soils.

The organic matter in topsoil and in subsoil differs in quantity and in composition (table 3). The total organic matter in the topsoil is 4.5 per cent; in the subsoil, 1.95 per cent. Also, the topsoil contains 4.4 times as much

water-soluble material and 2.5 times as much lignin as the subsoil. The elasticity of the topsoil is probably due to the large percentage of organic matter and specifically the lignin content. It is, therefore, the organic matter which gives to the topsoil its predominant characteristic. The amount and type of organic matter contained in the subsoil does not appear to be of a character to make a specific imprint on the physical characteristics of the soil. The extent of the influence of the organic matter on the latter is, therefore, difficult to judge.

Properties of the colloidal material separated from the soils

From the two soils the fractions < 0.002 mm. were separated, after dispersion in diluted NaOH solution, by means of a Sharples ultracentrifuge. The separated fine fraction was then electrodialyzed to obtain the H-clays. The material from the topsoil was very black, indicating a high content of

TABLE 4
Analysis of clays from Argentina topsoil and subsoil

	CLAY FROM TOPSOIL	CLAY FROM SUBSOIL
SiO ₂	3.1	3.6
R ₂ O ₃		
SiO ₂	5.7	7.4
Al ₂ O ₃		
SiO ₂	6.8	7.2
Fe ₂ O ₃		
Base-exchange capacity		
Potentiometric titration..... <i>m.e./100 gm.</i>	80	34
Conductometric titration..... <i>m.e./100 gm.</i>	84	39
Organic matter..... <i>per cent</i>	10.32	1.96
Heat of wetting..... <i>cal./gm.</i>	9.1	11.7

organic matter. The subsoil fraction was dark gray. These materials were analyzed for their content of silica and sesquioxides, organic matter, and base-exchange capacity. The data obtained are given in table 4.

According to these data, the clay from the topsoil, despite its small silica-sesquioxide ratio, possesses a much higher base-exchange capacity than the clay from the subsoil. This fact is very significant and points, on one hand, to the marked influence of the organic matter in the topsoil toward an increase of the base-exchange capacity, as already demonstrated by Baver (3); on the other hand, it indicates a relatively low base-exchange capacity of the inorganic colloid, which is remarkable in view of the high silica-sesquioxide ratio, especially of the colloid from the subsoil. Part of this silica, however, may be present as finely dispersed quartz. With this high silica-sesquioxide ratio goes a relatively high heat of wetting. A low base-exchange capacity is usually connected with a low cohesion, under comparable conditions of particle

size and water content; a high heat of wetting indicates a great affinity for water. These two properties indicate a material which is very susceptible

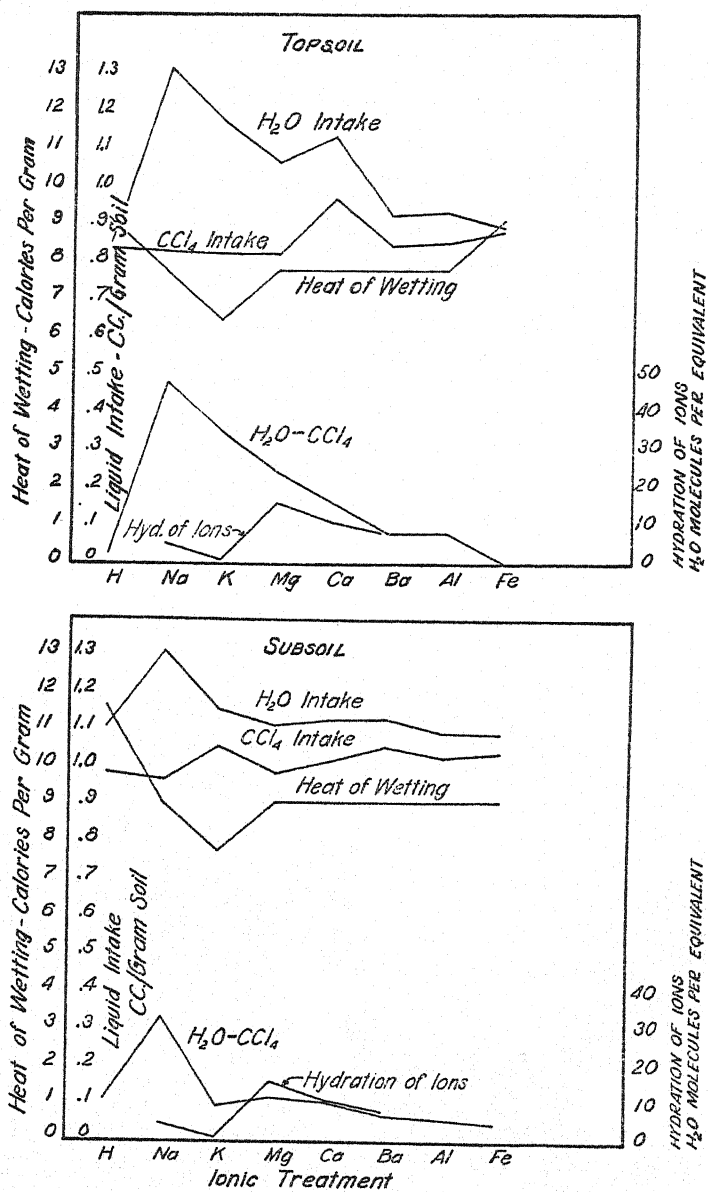


FIG. 2. PROPERTIES OF COLLOIDS FROM TOPSOIL AND SUBSOIL

to the action of water and which is a very troublesome material for a road subgrade. A soil possessing this type of clay can be expected to possess

properties more like those usually connected with a silty material than those usually attributed to the clays. As a whole, the clay of the subsoil appears to be of a freakish and undesirable nature.

Properties of the homoionic clays

From the hydrogen clays other homoionic modifications were prepared by neutralization with NaOH, KOH, $\text{Ca}(\text{OH})_2$, $\text{Mg}(\text{OH})_2$, $\text{Ba}(\text{OH})_2$ and by treatment with solutions of FeCl_3 and $\text{Al}_2(\text{SO}_4)_3$, respectively, with subsequent removal of excess salts. These clays were tested for heat of wetting and for intake of water and of carbon tetrachloride. The results of these tests are shown in figure 2.

It is remarkable that, for the clays of both topsoil and subsoil, the heat of wetting is only slightly sensitive to the exchange ions present. This might indicate that a large part of the heat of wetting is derived not from the hydration of the ions but rather from other hydration reactions on the surface of the particles. The curves showing the intake of water and of carbon tetrachloride for the clays of both topsoil and subsoil run parallel to a certain extent; the exceptions concern the Na- and K-clays of the surface soil and the Na-clay of the subsoil. These exceptions represent osmotic swelling. The curves representing the differences between intake of water and intake of carbon tetrachloride are of the same type as those for the hydration of the ions, if the osmotic swelling of the monovalent clays is taken into account.

CONCLUSIONS

From the data obtained it appears that the dominant factor of the topsoil is its elasticity, caused by the amount and type of organic matter available. Treatment with Na ion is very effective in reducing this property.

The most important property of the subsoil is its great avidity for water. This avidity appears to be correlated not so much with the exchange ions as with the surface of the soil particles as such. The effect of ionic substitution on the physical soil properties is not likely, therefore, to be very marked. However, greater effects can be expected by the presence in the soil of ions in excess of its exchange capacity.

REFERENCES

- (1) ANDERSON, M. S., AND BYERS, H. G. 1933 Character and behavior of organic soil colloids. U. S. Dept. Agr. Tech. Bul. 377.
- (2) BAVER, L. D. 1928 Relation of exchangeable cations to the physical properties of soils. *Jour. Amer. Soc. Agron.* 30: 930.
- (3) BAVER, L. D. 1930 Effect of organic matter upon several physical properties of soils. *Jour. Amer. Soc. Agron.* 32: 703.
- (4) HOGENTGLER, C. A., WINTERMYER, A. M., AND WILLIS, E. A. 1931 Subgrade soil constants, their significance and their application in practice. *Pub. Roads*, 12, nos. 4 & 5.
- (5) KÖGLER, F. 1938 Baugrund und Bauwerk. Wilhelm Ernst & Sohn, Berlin.
- (6) WAKSMAN, S. A. 1938 Humus, ed. 2. Williams & Wilkins Company, Baltimore.

- (7) WAKSMAN, S. A., AND HUTCHINGS, I. J. 1935 Chemical nature of organic matter in soils. *Soil Sci.* 40: 347.
- (8) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat. *Soil Sci.* 26: 113.
- (9) WINTERKORN, H. F. 1936 Surface behavior of bentonites and clays. *Soil Sci.* 41: 25-32.
- (10) WINTERKORN, H. F. 1936 Surface-chemical factors influencing the engineering properties of soils. *Proc. Highway Res. Bd.* 1936: 294.
- (11) WINTERKORN, H. F., AND BAYER, L. D. 1934 Sorption of liquids by soil colloids: I. *Soil Sci.* 38: 291-298.
- (12) WINTERKORN, H. F., AND ECKERT, G. W. 1940 Consistency and physicochemical data of a loess pampaneo soil: I. *Soil Sci.* 49: 73-82.

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